MEDIZINISCHE UNIVERSITÄT
INNSBRUCK

PROGRAM AND ABSTRACT BOOK

3RD MUI-START SYMPOSIUM

MUI-START SYMPOSIUM
Wednesday 15th October 2014, Center of Chemistry & Biomedicine

PROGRAM

15TH OCTOBER, 2014; CENTER OF CHEMISTRY & BIOMEDICINE, INNRAIN 80
14:30 H – 17:30 H

TIME	TITLE	Abstract
14:30 – 14:40	**Welcome Address of Prof. Christine Bandtlow (VR Research and International Affairs)**	
	Oral presentations	
14:40 – 14:50	**Mag. Ulrike Binder PhD** "*Galleria mellonella* as a host model to study invasive fungal infections due to Mucorales"	O 01
14:50 – 15:00	**Mag. Michael Blatzer PhD** "BoIA a transcriptional regulator required for stress adaptation in *A. fumigatus*?"	O 02
15:00 – 15:10	**DI (FH) Dr. Judith Hagenbuchner** "Regulation of glycolysis and mitochondrial respiration by BIRC5/Survivin in neuronal tumor cells"	O 03
15:10 – 15:20	**Assistenzprofessor Dr. Joachim Schmutzhard** "Sepsis otopathy: proof of concept"	O 04
15:20 – 15:30	**James Wood PhD** "Dopaminergic modulation at synapses of the medial central amygdala"	O 05
15:30 – 15:40	**Dr.med.univ. Gert Klug** "Fetuin-A: Relation to myocardial function and left ventricular remodeling after acute ST-segment elevation myocardial infarction"	O 06

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Poster introductions ***15:40 h - 16:10 h***	**Abstract**
Dr.med.univ. Ramon Tasan "Characterization of Neurokinin B neurons in the amygdala and their role in anxiety and fear"	P 01
Dr. Kerstin Siegmund "Coronin 1A - an essential modulator of T cell-intrinsic functions?"	P 02
Dr.med. Elke Griesmaier "PRE-084, a sigma-1 receptor ligand, protects against excitotoxic perinatal brain injury"	P 03
Mag. Dr.rer.nat. Christa Pfeifhofer-Obermair "PKC Controls IL-10 secretion in macrophages during protective immunity against infection with *Salmonella enterica* Serovar *Typhimurium*"	P 04
Dr.med.univ. Hannes Neuwirt PhD "Complement system and MAPK signaling in calcineurin-inhibitor induced nephrotoxicity"	P 05
OA Dr. Gregor Brössner "Noninvasive measurement of brain temperature in magnetic resonance imaging"	P 06
Dr. Michiel Langeslag "FABRY pain: understanding pain in FABRY disease"	P 07
Dr.rer.nat. Oliver Schmidt "Identification and characterization of an 'endosomal stress response' pathway"	P08
Cedric Hubert De Smet PhD "A novel lipid sensor in the endosomal membrane"	P 09
Mag. Martin Bodner PhD "Helena, the hidden beauty: resolving the most common West Eurasian mtDNA control region haplotype at the highest resolution by massively parallel sequencing "	P10

Dr.rer.nat. Sebastian Herzog "Regulation of B cell development by long non-coding RNAs"	P11
Coffee break ***16:10 h -16:25 h***	

Short Project Overviews ***16:25 h – 16:45 h***	**Abstract**
Dr.rer.nat Laura Zamarian "Decision making abilities in patients with multiple sclerosis – Assessment and training"	S 01
Dr. med. Astrid Grams "Intracranial aneurysm as a hypertensive disease"	S 02
Dr. Daniela Kuzdas-Wood "Cardiovascular phenotyping of a transgenic mouse model for multiple system atrophy"	S 03
Dr.rer.nat. Natalia Schiefermeier "microRNAs in axonal regeneration: regulation of mir-138 and mir-21 by gp130 signaling in peripheral nerve injury and recovery"	S 04
Dr.med.univ. Gabriele Von Gleissenthall " Tryptophan and kynurenine metabolism in alcohol dependent patients in acute and medium-term withdrawal "	S 05
Mag.rer.nat. Martin Puhr PhD "Identification and assessment of altered miRNA expression profiles to improve early prostate cancer detection"	S 06
Mag.rer.nat. Mario Gründlinger PhD "Peroxisomal import pathways and their role in *A. fumigatus* virulence and adaptation"	S 07
Dr.med.univ. Isabel Heidegger PhD "The role and impact of the tumor endothelial regulator "Robo4" in prostate cancer"	S 08

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Dr.rer.nat. Johanna Gostner "Formaldehyde metabolism – On the role of formaldehyde in inflammation"	S 09
Lourdes Rocamora Reverte PhD "GC production in the thymus and its influence on T cell development"	S 10
Mag. Dr. Phil. Luca Fava "Caspase-2 in cell death induced by polyploidization"	S 11
Mag.rer.nat Ingo Bauer PhD „AN4022–A novel HDAC complex component as basis for a novel antifungal therapy"	S 12
Coffee and Poster discussion ***16:45 h – 17:30 h***	

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Oral presentations

Oral presentations should last no more than 7 min to have 3-5 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 mounted previously on the stands placed outside in the corridor. Please mount the posters according to the numbers in the program and which will be also posted on the stands.

The project leaders will first give a short introduction (1 min) on the poster topic using a PowerPoint slide as support.

The aim of this 1-minute-introduction is to draw the attention of the participants to the posters and promote further discussion at the stands.

Short Overview of the projects funded under the 5th MUI-START call

The leaders of the recently funded projects will present themselves and give a short overview (1 min) on the topic of their projects using a PowerPoint slide as support.

Please send your PowerPoint presentation or slide to the Servicecenter-Forschung (maria.perez@i-med.ac.at) by the **14th of October**.

ABSTRACTS

Oral Presentation

O 01: *Galleria mellonella* as a host model to study invasive fungal infections due to Mucorales

Ulrike Binder

Division of Hygiene and Medical Microbiology, Medical University of Innsbruck, Austria

Background: Mucorales are increasingly causing infections in immunocompromised patients. However, little is known about the relation between different members of Mucorales and their virulence potential. The aim of this study was to determine whether Galleria mellonella larvae are useful (i) as an in vivo infection model for Mucorales; (ii) to compare virulence potential of Lichtheimia spp., Rhizopus spp., Rhizomucor pusillus and Mucor circinelloides, and check for correlation to physiological attributes; and (ii) for evaluating the in vivo efficacy of liposomal amphotericin B, posaconazole, isavuconazole and nystatin-intralipid. Furthermore, in vivo outcome was compared to in vitro susceptibility data.

Methods: G. mellonella larvae were infected with different concentrations of clinically relevant Mucorales and survival was recorded over a 144 h period. Survival rates were correlated to physiological. Efficacy of antifungals was evaluated with administration of treatments 2 h post infection.

Results: G. mellonella larvae were sensitive to Mucorales infection in a dose- and temperature-dependent manner. Virulence was dependent on vitality of inoculated spores, as heat-killed spores had no detrimental effect on survival. Furthermore, histological examination of infected larvae showed that fungi killed larvae via active infection mechanism. Pathogenicity could be linked to the clinically relevance, since Rhizopus arrhizus, Mucor ciricnelloidies and Rhizopus microsporus exhibited highest virulence. Virulence potential correlated with species-dependent physiological attributes, such as growth speed, spore size and oxidative stress tolerance.

Evaluation of antifungal efficacy in vivo demonstrated a drug- and member dependent outcome. Nystatin-intralipid exhibited best activity against Mucorales, followed by posaconazole, while limited efficacy was seen for amphotericin B and isavuconazole.

Conclusion: The findings of this study proof, that the invertebrate model G. mellonella is a useful tool to study virulence and putative treatment options of Mucorales.

Oral Presentation

O 02: BolA - a transcriptional regulator required for stress adaptation in *Aspergillus fumigatus*?

Michael Blatzer[1] and Hubertus Haas[2]

[1]Division of Hygiene and Medical Microbiology, Medical University of Innsbruck, Austria
[2]Division of Molecular Biology, Biocenter, Medical University of Innsbruck, Austria

Background: The BolA protein family is widespread among eukaryotes and bacteria. BolA was previously defined as a transcriptional regulator only based on structural evidence indicating the presence of helix-turn-helix motif typical of nucleic acid-binding proteins. In *E. coli*, BolA causes a spherical shape and is overexpressed during oxidative stress. Maintenance of optimal iron levels inside the cell is critical for all eukaryotes and most prokaryotes, as iron is both essential and, in excess, potentially toxic. Therefore, cells must be able to sense iron levels and maintain iron homeostasis with sufficient yet non-toxic levels of this key nutrient. Iron-sensing is best characterized in *S. cerevisiae,* where the transcription factor Aft1 senses mitochondrial Fe-S cluster biosynthesis activity involving the monothiol glutaredoxins (Grx3/4) and the BolA protein Fra2. Non-Saccharomyces fungal species, including *Aspergillus fumigatus*, lack Aft1 homologs and control iron homeostasis using different transcription factors, homologs of *A. fumigatus* SreA and HapX. Nevertheless, most fungal species possess Fra2 homologs but information on their functions is lacking. Here we elucidate the possible role of the *A. fumigatus* BolA homolog in heme and iron metabolism.
Methods: The function of BolA in *A. fumigatus* was studied by deletion and reconstitution of the encoding *bolA* gene with a GFP-tagged version followed by phenotyping of the strains under different growth conditions and subcellular localization.
Results: BolA-deficiency decreases siderophore production and growth during iron starvation. Remarkably, the growth defect is most pronounced at ambient temperatures and partly cured under hypoxic conditions or elevated incubation temperatures. Consistent with the mitochondrial localization of BolA, strains lacking bolA display elevated protoporphyrin IX levels and decresed heme levels.
Conclusion: BolA-deficiency causes severe growth defects and affects susceptibility to various external stressors. Siderophore and heme levels are decreased in strains lacking BolA indicating a central function of BolA in heme and iron homeostasis.

Oral Presentation

O 03: Regulation of glycolysis and mitochondrial respiration by BIRC5/Survivin in neuronal tumor cells

Judith Hagenbuchner
Department of Paediatrics II, Medical University of Innsbruck, Austria

Background: Adverse forms of neuroblastoma (NB), a childhood malignancy that develops from immature neuronal progenitor cells frequently carry a gain of chromosome 17q, which leads to overexpression of the anti-apoptotic protein BIRC5/Survivin. Survivin is repressed by FOXO3, a transcription factor that is activated in response to genotoxic stress or growth factor withdrawal. We have recently shown that FOXO3 triggers biphasic ROS accumulation and subsequent apoptosis in neuronal cells. Interestingly, high endogenous levels of Survivin efficiently prevent these ROS waves and apoptotic cell death. To investigate the molecular basis of ROS- and apoptosis inhibition by Survivin the project was designed to analyze the effects of Survivin on mitochondrial architecture, mitochondrial respiration and energy metabolism in neuronal tumor cells. This suggests that glycolysis-inhibitors target an “archilles heel” of Survivin-overexpressing NB and may be highly useful as chemosensitizers in the treatment of high-stage NB.
Methods: The project was performed with different high and low stage neuroblastoma cell lines varying in their Survivin expression level. Mitochondrial structure was analyzed by live cell microscopy, the expression of fusion/fission proteins as well as components of the respiratory chain is assessed by immunoblot. Respiration and activity of single respiratory complexes was measured in collaboration with PD. Dr. Andrey Kuznetsov at the Cardiac Research Laboratory. The effect of glycolysis inhibitors on viability and physiological activity of Survivin-overexpressing or Survivin-knock-down cells (generated via retroviral and lentiviral infection) was analyzed via flow cytometry, cell viability assays, Glucose-, Lactat- and ATP-measurements and in the last part *in vivo* in a xenograft mouse model.
Results: We were able to show that Survivin levels define a threshold for the sensitivity of neuroblastoma cells to glycolysis inhibitors and that Survivin’s apoptosis inhibitory function depends on its mitochondrial localization and the recruitment of the fission protein Drp-1. Thereby increased Survivin expression leads to reduced activity of respiration complex I and increased glycolyis. (Hagenbuchner et al, Oncogene, 2013 - Sanofi Preis Medical University
Innsbruck 2014). The experiments suggest that glycolysis inhibitors lead to proteasome-independent degradation of mitochondrial Survivin, which sensitizes for chemotherapeutic agents. This occurs most likely through induction of autophagy, as glycolysis inhibitor-induced Survivin degradation is associated with increased conversion of LC3-I to LC3-II and can be prevented by knock-down of the mitophagy-related ubiquitin-ligase Parkin. The therapeutic potential of glycolysis-inhibitors was also assessed *in vivo* in a xenograft mouse model.
Conclusion: The current data *in vitro* and *in vivo* suggests that glycolysis-inhibitors target an “archilles heel” of Survivin-overexpressing NB and are highly useful as chemosensitizers in the treatment of high-stage NB. This is also therapeutically important as the hexokinase-inhibitor LND is already in use in phase II and III clinical trials for breast, ovarian and non-small-cell lung ~~carcinoma~~ and 2DG showed promising effects in combination with standard therapeutics for the treatment of glioblastoma multiforme patients. Our results therefore suggest glycolysis-inhibitors as promising agents to overcome the chemotherapy-protective effect of Survivin in poor-prognosis, high-stage neuroblastoma.

Oral presentation

O 04: Sepsis otopathy: proof of concept

Joachim Schmutzhard

University Clinic for Ear, Nose and Throat Medicine; Medical University of Innsbruck, Austria

Background: Critical Care medicine has been associated with hearing impairment. Multiple causes have been identified. With 3 out of 1000 per year sepsis is a frequent cause for critical care treatment. So far sepsis has not been linked to hearing loss. The aim of this project was to examine the effect of lethal and survived sepsis to hearing. Furthermore the effects of different clinical sepsis parameter on the inner ear were examined with an in vitro approach.

Methods: Experimental sepsis in C57 Bl/6 has been established using cecal ligation puncture technique. An objective hearing test has been done using auditory brainstem responses. Hearing was tested before sepsis induction and at a second time point. The first approach included 44 animals with a severe 100 % lethal form of sepsis. The second measurement was done at the peak of the disease. Furthermore the temporal bones were examined using electron microscopy and immunohistochemistry for apoptosis marker.

The second approach included 50 animals, with a mild induction of sepsis anticipating 50 % survivors. The hearing threshold and the histomorphology for apoptosis marker of the surviving animals were evaluated on day 7.

For the third experiment a whole organ culture of the adult murine cochlea was established. The clinical sepsis parameter E.Coli positive blood culture, hypothermia and lactateazidosis, which have been measured in the first experiment, were applied to the murine cochlea under culture conditions. The inner ears were further evaluated for apoptosis marker, cytoskeletal marker and neuronal markers.

Results: The first experiment revealed a significant hearing loss in the sepsis group. The immunohistochemical evaluation showed an up regulation of apoptosis marker in the supporting cells in the organ of Corti. Furthermore, vacuolization at the basis of the inner hair cells could be found in the electronmircroscopy, indicating glutamate excitotoxicity.

The second experiment showed that significant hearing loss could also be found in animals surviving sepsis. The immunohistochemical evaluation showed an up-regulation of apoptosis marker in the inner ears of the hearing impaired animals. On the one hand pathologic alterations could be found in the supporting cells and on the other hand in the spiral ligament.

The third approach using an in vitro setting revealed that acidosis and elevated lactate level lead to an induction of apoptosis in the spiral ligament and the organ of Corti. Furthermore a down regulation of the cytoskeletal markers and the neuronal markers could be found in this group.

Conclusion: Sepsis leads to a significant hearing loss. Furthermore the physiologic impairment could be linked to an induction of apoptosis and glutamate excitotoxicity at the inner hair cell. The pathologic effect could also be shown on animals surviving the disease. These in vivo observed changes could be connected with an in vitro set up to lactate acidosis.

Oral Presentation

O 05: Dopaminergic modulation at synapses of the medial central amygdala

James Wood
Neuropharmacology Group, Department of Pharmacology, Medical University Innsbruck, Austria

Background: Anxiety disorders and post-traumatic stress disorder (PTSD) are thought to arise from maladaptive alterations in brain circuits involved in fear and survival responses. In particular, impairments in extinguishing aversive memories are believed to contribute to anxiety disorders and PTSD. Synaptic plasticity in a circuit involving the basolateral amygdala (BLA), main intercalated nucleus (Im) and medial subdivision of the central amygdala (CeM) has a central role in fear extinction. Specifically, feed-forward inhibition between the BLA and CeM, via GABAergic neurons of the Im, is enhanced after successful fear extinction. Dopaminergic inputs to the amygdala provide essential cues for processing affective stimuli; however, how dopamine modulates the activity of the BLA-Im-CeM circuit is unclear. Thus, the aim of the present study was to characterize the expression of dopamine receptors in the BLA, Im and CeM and determine whether dopamine influences feed-forward inhibition between the BLA and CeM.
Methods: Feed-forward inhibition between the BLA and CeM was investigated by performing whole-cell patch clamp recordings in acute brain slices containing the amygdala. Evoked inhibitory postsynaptic potentials (IPSPs) were measured in CeM neurons in response to electrical stimulation of the BLA in the presence of dopamine (50-100 µM) with or without the D1 receptor antagonist SCH 23390 (10 µM). Immunohistochemistry was performed to determine the distribution of dopamine receptors in the BLA-Im-CeM circuit.
Results: Immunohistochemical characterization revealed expression of dopamine D1 receptors in the Im but not in the CeM or BLA. Dopamine application reversibly suppressed feed-forward inhibition of the CeM by 61% (baseline IPSP: -18.3 ± 2.6 mV, +dopamine IPSP: -6.9 ± 4.1 mV, $p < 0.01$). SCH 23390 did not block the dopamine-mediated suppression of feed-forward inhibition indicating involvement of non-D1 receptors.
Conclusion: Our results demonstrate that dopamine suppresses feed-forward inhibition between the BLA and CeM, a circuit critically involved in the process of fear extinction. Thus, pharmacological interventions of the dopamine system may support extinction therapy in anxiety disorders and PTSD. In future studies I will determine which dopamine receptors mediate suppression of feed-forward inhibition in the BLA-Im-CeM circuit.

Oral Presentation

O 06: Fetuin-A: Relation to myocardial function and left ventricular remodeling after acute ST-segment elevation myocardial infarction

Hans-Josef Feistritzer[1] Gert Klug[1], Sebastian Reinstadler[1], Johannes Mair[1], MT Gröber[1], Michael Schocke[2], Wolfgang-Michael Franz[1] and Bernhard Metzler[1]

[1]University Clinic of Internal Medicine III, Medical University of Innsbruck, Austria
[2]University Clinic of Radiology, Medical University of Innsbruck, Austria

Background: Fetuin-A, a glycoprotein synthesized by the liver, increases the solubility of calcium and phosphorus and plays a key role in anti-inflammatory processes. The relationship between circulating fetuin-A and cardiac remodelling has not been studied so far in STEMI patients. We therefore investigated the association between plasma fetuin-A concentrations and left ventricular function, infarct size and the occurrence of adverse remodelling at 4 months after mechanical reperfusion for STEMI.
Methods: All patients (n = 52) underwent contrast-enhanced cardiac magnetic resonance imaging within the first week after STEMI and 4 months thereafter. Left ventricular dimensions and function were measured from cine true-FISP sequences. Infarct size was determined with the use of late gadolinium enhanced images. Fetuin-A levels were determined from blood samples drawn at a median of 2 days (IQR 1 - 3 days) after STEMI by a sandwich immunofluorescent assay. Adverse remodelling was defined as an increase in end-diastolic volume of ≥ 20% after 4 months.
Results: Fetuin-A levels (mean: 700 ± 195 µg/ml) were significantly related with 4-months ejection fraction ($r = 0.409$, $p = 0.002$) and trended to correlate with baseline ejection fraction ($r = 0.236$, $p = 0.092$). Patients with adverse remodelling (n = 7) showed significantly lower baseline fetuin-A levels (528 ± 88 µg/ml vs 737 ± 190 µg/ml , $p < 0.001$) compared to patients without remodelling (n = 45). The area under the curve of fetuin-A (0.79, 95% CI 0.67 to 0.92) with the optimal cut-off value of 670 µg/ml revealed 100% sensitivity and 67% specificity (PPV = 32%, NPV = 100%) in the prediction of adverse remodelling at 4 months follow-up. Fetuin-A levels were not associated with baseline or 4 months infarct size.
Conclusion: Circulating fetuin-A at day 2 after STEMI is a potential predictor of 4-months myocardial function and adverse remodeling. These findings highlight the possible role of fetuin-A as a robust biomarker predicting outcome after reperfused STEMI.

Poster Presentation

P 01: Characterization of Neurokinin B neurons in the amygdale and their role in anxiety and fear

Ramon Tasan

Detpartment of Pharmacology, Medical University of Innsbruck, Austria

Background: Anxiety disorders constitute a major burden for the society and are characterized by pathological expression of anxiety and fear. Among the different brain areas involved in the encoding and modulation of fear memories the amygdala plays an exceptionally important role. Intrinsic and extrinsic amygdala connections are predominantly mediated by glutamate and GABA, but the resulting behavioral response is fundamentally shaped by various neuromodulators, including different neuropeptides. Despite extensive investigations, the role of many of these neuropeptides is still poorly understood. Among the neuropeptide family of tachykinins, substance P is promoting anxiety-related behavior, whereas the role of neurokinin B (NKB) that is abundantly expressed in different amygdala nuclei is not clear yet. Thus, we wanted to investigate the role of NKB and NKB-expressing neurons in the amygdala in fear conditioning and extinction.
Methods: To test the specific role of NKB-expressing neurons in the basolateral amygdala (BLA), Tac2-Cre mice were locally injected into the BLA with a Cre-dependent rAAV-vector expressing tetanus-light-chain (TeLC) or rAAV-hM3Dq for specific and local permanent silencing or for transiently activating NKB neurons, respectively, followed by *Pavlovian* fear conditioning two weeks later. *Pavlovian* fear conditioning was used as a simple form of associative learning by pairing a tone with a mild electric food shock. Fear extinction was performed the following day by repetitive exposure to the tone without foot-shock. Furthermore, immunohistochemical and electrophysiological methods were used to characterize NKB-expressing neurons in the BLA in more detail.
Results: Inhibition of NKB-expressing neurons in the BLA did not affect fear acquisition or context fear but resulted in reduced expression of tone-induced freezing and facilitated extinction learning. This change was also observed in extinction recall, demonstrating a persistent effect of NKB neuron inhibition in the BLA. Interestingly, inhibition of all GABA-ergic neurons in VGAT-Cre mice resulted in increased fear and delayed extinction, indicating a highly specific function of NKB neurons in the BLA. NKB neurons in the BLA were GABA-ergic and co-localized with the calcium binding protein calretinin and vasoactive intestinal peptide (VIP).
Conclusion: Together our data indicate that inhibition of NKB-expressing neurons in the BLA reduces the expression of fear while facilitating fear extinction. Further studies will clarify the exact mechanism and the contribution of NKB itself in this process.

Poster Presentation

P 02: Coronin 1A - an essential modulator of T cell-intrinsic functions?

Kerstin Siegmund[1], Jean Pieters[2] and Gottfried Baier[1]

[1]Department for Pharmacology and Genetics, Division of Translational Cell Genetics, Medical University of Innsbruck, Austria
[2]Biozentrum, University of Basel, Switzerland

Background: Coronin 1A (Coro1A), a member of the evolutionary conserved coronin protein family, is highly expressed in all leukocytes. In mice and human, genetic inactivation of *coro1a* results in immuno-deficiencies that are linked to a strong reduction of naïve T cell numbers in peripheral organs. However, despite its essential role in T cell survival, migration and T cell receptor signaling, it is currently unclear how Coro1A regulates these tasks on a molecular level. Furthermore, all results published so far have been obtained with conventional *coro1a* knockout mice or mice bearing spontaneous or induced germ line mutations of *coro1a* – leading to absence of Coro1A in all leukocyte subsets. Therefore, the anticipated T cell intrinsic and furthermore a potential T cell subset-specific function of Coro1A remain to be proven.

Methods: Wild-type and *coro1a*-deficient mice were subjected to experimental autoimmune encephalomyelitis (EAE), a murine model of multiple sclerosis, or acute hepatitis was induced by intravenous Concanavalin A (ConA) injection. Disease severity was determined by body weight, disease score (EAE) and serum levels of liver enzymes (ConA-hepatitis) as well as histology. T cell-specific cytokine expression was analyzed *ex vivo* by flow cytometry or ELISA. Effector and regulatory T (Treg) cells isolated from wild-type and *coro1a*-deficient mice were analyzed using *in vitro* cultures. In addition, wild-type and *coro1a*-deficient dendritic cells (DC), differentiated *in vitro* were compared for their capacity of induce T cell proliferation. To elucidate the T cell-intrinsic role of Coro1A mice baring a *coro1a* allele flanked by loxP sites (coro1a$^{fl/fl}$) were crossed to transgenic mice that contain the CD4 enhancer, promoter and silencer sequences driving the expression of Cre ("CD4[Cre]").

Results: Conventional *coro1a*-deficient mice, harboring strongly reduced T cell number, were resistant to EAE induction. While disease susceptibility could be restored by adoptive transfer of high number of wild-type T cells, depletion of Treg cells - fully functional to suppress proliferation of their own CD4$^+$ T cells *in vitro* - had no impact on EAE induction. However, *coro1a*-deficient mice develop signs of acute hepatitis similar than wild-type mice after administration of ConA; despite impaired activation of T cells in the absence of Coro1A. Using *in vitro* and *in vivo* approaches we could show that wild-type and *coro1a*-deficient DCs are equally potent to induce antigen-specific proliferation of wild-type T cells. The first offspring of our coro1a$^{fl/fl}$ x CD4[Cre] breeding are now available for further analyses.

Conclusion: *In vitro* and *in vivo* analyses performed thus far strongly suggest that Coro1A is key player in T cell survival and function and that this is due to a T cell-intrinsic role of Coro1A. While important for naïve T cell maintenance, Coro1A seems to be dispensable for other leukocyte function during acute liver inflammation induced by ConA. With the T cell-specific *coro1a* knockout mouse (coro1a$^{fl/fl}$ x CD4[Cre]), generated recently in our laboratory, we have now a genetic tool available to address any T cell-specific role of Coro1A.

Poster Presentation

P 03: PRE-084, a sigma-1 receptor ligand, protects against excitotoxic perinatal brain injury

Elke Griesmaier[1], Katharina Stock[1], Martina Urbanek[1], Martin Hermann[2], Anna Posod[1], Ruslan I. Stanika[3], Gerald J. Obermair[3] and Ursula Kiechl-Kohlendorfer[1]

[1]Department of Paediatrics II, Neonatology, Innsbruck Medical University, Austria
[2]Department of Anaesthesiology and Critical Care Medicine, Innsbruck Medical University, Austria
[3]Department of Physiology and Medical Physics, Innsbruck Medical University, Austria

Background: Prematurity is a major determinant of neonatal mortality and morbidity and the number of preterm birth is still on the rise. Our group focuses on substances acting as sigma-1 receptor agonists, which have been shown to be neuroprotective in adult and newborn animal models of brain injury. Since sigma-1 receptor agonists are already undergoing clinical trials in adult neurological diseases, they might be considered a promising therapeutic option also in preterm brain injury. We have previously shown that the selective sigma-1 receptor agonist PRE-084 (2-(4-morpholinethyl)1-phenylcyclohexane-carboxylate) protects against neonatal excitotoxic brain injury in vivo. The aim of the present study was to investigate whether PRE-084 is able to prevent neurotoxicity following glutamate exposure in vitro.

Methods: OLN-93 cells (oligodendroglial cell line) were pre-treated with PRE-084 before glutamate (3mM) was applied and subsequently analysed by using a cytotoxicity assay (CCK-8). In OLN-93 cells PRE-084 was applied in three dosages (1, 10 and 100µM) prior to glutamate.

Primary hippocampal neurons were pre-treated with PRE-084 before glutamate (50µM) was applied and subsequently analysed for cell death (PI/calcein AM) using confocal microscopy. Neurons were randomly assigned to one of the following groups: i) control ii) glutamate iii) glutamate+PRE-084. PRE-084 was applied in two dosages (10 and 100µM) prior to glutamate.

Results: The application of PRE-084 had no effect on cell cytotoxicity in OLN-93 cells (PRE-084 1µM: 70.00 (66.75;74.75), 10µM: 76.00 (65.75;83.75) and 100µM 77.00 (60.00;85.75)) compared with the untreated glutamate control group (74.00 (67.75;78.50); n=6, $p>0.05$). The application of PRE-084 significantly reduced the number of dead cells (PRE-084 10µM: 22.09 (20.50;28.84) and 100µM 25.87 (18.77;33.40)) compared with the untreated glutamate control group (43.56 (39,86;46.02); n=3, $p<0.05$) in primary hippocampal neurons.

Conclusion: Our data show that administration of PRE-084 protects against glutamate-induced cell death in primary hippocampal neurons, but not in oligodendroglial OLN-93 cells. PRE-084 shows considerable promise of playing a role as therapeutic strategy in preterm brain injury and might provide an adequate means of combating this major cause of neurological disability in infancy. In ongoing studies we are investigating the underlying mechanisms more in detail.

Poster Presentation

P 04: PKC Controls IL-10 secretion in macrophages during protective immunity against infection with *Salmonella enterica* Serovar *Typhimurium*

C. Pfeifhofer-Obermair[1], M. Nairz[2], T. Gruber[1], S. Peer[1], N. Kleiter[1], V. Klepsch[1], G. Weiss[2], G. Baier[1]

[1]Department for Pharmacology and Genetics, Division of Translational Cell Genetics, Medical University of Innsbruck, Austria
[2]Department of Internal Medicine VI/Infectious Diseases, Immunology, Rheumatology, Pneumology, Medical University of Innsbruck, Austria

Background: *Salmonella enterica* serovar *typhimurium* (*S. typhimurium*) is a Gram negative, facultative intracellular bacterium, which invades and multiplicates within mononuclear phagocytic cells in liver, spleen, lymph nodes, and Peyer`s Plaques. *S. typhimurium* causes severe gastrointestinal disorders in humans and typhoid fever with systemic infections in mice. Macrophages, as one of the first barriers of the innate immune system, try to rapidly control *S. typhimurium*, however, these bacteria can evade immune control by macrophages and even multiply within these cells by mechanisms which are insufficiently understood so far.
Methods: The Protein Kinase C family is a serine-threonine kinase family required for full T cell activation as well as for adaptive immune responses. Wild type mice and mice deficient for PKC were infected with *Salmonella enterica* serovar *typhimurium*. Survival, serum cytokines, and colony forming units in liver and spleen were analyzed. As an *in vitro* model we either infected bone marrow derived macrophages with *S. typhimurium* or stimulated them with lipopolysaccharide (LPS) + interferon-gamma. The capacity to kill the bacteria after infection was analyzed by measuring colony forming units, and gene expression and protein levels of pro- and anti-inflammatory cytokines were analyzed.
Results: PKC-deficient mice fail to mount appropriate innate immune responses determined by a markedly decreased survival combined with a significantly enhanced number of bacteria in spleen and liver when compared to wildtype mice. This is paralleled by a significant increase in interleukin-10 serum levels in PKC-deficient mice which was also confirmed *in vitro* when challenging bone marrow derived macrophages with *S. typhimurium* or LPS + interferon-gamma.
Conclusion: Our data indicate that PKC are important to mount an appropriate immune response in macrophages challenged with Gram negative bacteria *Salmonella enterica* serovar *typhimurium* by regulating the production of the anti-inflammatory cytokine interleukin-10. The elucidation of underlying molecular mechanisms are in the focus of our current research.

Poster Presentation

P 05: Complement system and MAPK signaling in calcineurin-inhibitor induced nephrotoxicity

Beatrix Loeschenberger[1], Iris E. Eder[2], Martin Puhr[2], Michael Rudnicki[1], **Hannes Neuwirt**[1]

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Background: The gap between organ demand and supply to be used for transplantation is wide and getting wider. One way to improve the situation is to prolong allograft survival. One entity that significantly contributes to renal allograft loss is calcineurin inhibitor (CNI) nephrotoxicity (CIN). Various mechanisms are discussed to play a role in CIN pathogenesis; one of which is complement mediated injury.
Purpose: To investigate the impact of CNIs on MAPK signaling, complement regulators and complement activation.
Methods / **Results**: We have performed experiments utilizing two proximal tubule cell lines of human origin, HK2 and hTERT-RPTCs. 10µM cyclosporinA (CyA) and tacrolimus (FK506) treatment induced activation/phosphorylation of MAPK1/-2 in both cell lines.
This was associated with a significant decrease in protein levels of suppressor of cytokine signaling (SOCS)-3, which we have shown recently to act as a negative regulator of MAPK1/-2 signaling. Hence, one might hypothesize that SOCS-3 downregulation enhances MAPK activation.
In order to investigate the regulation of complement regulatory proteins DAF (CD55) and MCP (CD46), which inhibit or accelerate disassembly of C3 convertase and inhibit C3b and C4b, we assessed protein expression levels by Western Blotting and found that CNI treatment caused a down regulation of DAF and MCP. Next we overexpressed and knocked down SOCS-3 via transient transfection with an overexpression plasmid (…) and specific siRNA. Under these SOCS-3 modulations we found that MAPK-activation and DAF/MCP regulation is strongly influenced by SOCS-3 expression levels. Thus, one might speculate that a part of CIN may be attributed to dysregulated complement system activation by the SOCS-3-MAPK1/-2 signaling complex, which induces down regulation of complement regulators DAF/MCP.
Finally, we measured cellular proliferation by 3H-thymidine incorporation in cells treated with CsA and FK506 in heat-inactivated normal human serum (HIS) and compared it with the effects in normal human serum (NHS). Interestingly, cellular growth with HIS was approx. 20% better than with NHS. Thus, reduced cell growth could be attributed to enhanced complement system induced cell death and therefore may be of relevance for development of CIN.
Conclusion: Based on our data, we hypothesize that MAPK-activation by CNI, which enhanced by CNI-induced SOCS-3 downregulation, yields downregulation of DAF and MCP and finally complement activation on human proximal tubule cells. This might be one additional pathomechanism of CIN development.

Poster Presentation

P 06: Non-invasive measurement of brain temperature in magnetic resonance imaging

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Background: Today monitoring of brain temperature has become a major focus of scientific interest since (1) fever has been recognized as a significant player in patients with acute neuronal injury, (2) fever has been shown to be an independent predictor of mortality and morbidity in various disease entities and (3) in recent years efficacious devices have been developed to control body core temperature or even achieve hypothermia in clinical routine.
Unfortunately, with the exception of Magnetic Resonance Spectroscopy (MRS) and NIRS, only invasive techniques enable measurement of brain temperature requiring neurosurgical intervention for probe insertion. Preliminary experimental studies in phantoms and experimental models have shown close correlations between temperature as measured by MRS and implanted probes
Furthermore, Magnetic resonance imaging provides a non-invasive approach that perfectly combines temperature measurement with high spatial resolution of the brain. Therefore, MRS represents a promising method in Non-Invasive Thermometry by using the water to chemical shift.
Methods: The chemical shift of nuclei in Magnetic Resonance Spectroscopy Data is due to changes in larmor frequency generated by local differences in the electronic structure. Protons involved in hydrogen bonds are highly susceptible to changes in temperature thus leading to alterations in resonance frequency. To quantify these changes the shift of the water peak is compared to a temperature-independent reference molecule. In a first step we are measuring the chemical shift in a double-spherical phantom. The inner sphere is filled with our test solution composed of N-acetylaspartate, choline and creatine solved in distilled water. We use two differently concentrated test solutions with 25mM and 50mM respectively. The outer sphere is filled with water, heated up to 51.4° Celsius, in order to adjust the temperature within the inner sphere. A LumaSense Luxtron m600 fluoroptic probe is placed in the test solution for real-time temperature measurements. While the test solution is progressive cooling we perform the scans at predefined time points. In a second step we acquire data derived from healthy volunteers using MRS, with simultaneous non-invasive surface temperature measurement, to obtain data for building our reference curve.
The data is acquired on a Siemens MAGNETOM Verio 3 Tesla MRI Scanner using a 32 channel head coil. We choose to perform spectroscopy with Single Voxel Spectroscopy technique and Chemical Shift Imaging technique. We define the Volume Of Interest (VOI) to be 10x10x10 and apply weak water suppression. For data analysis we use the interface jMRUI based on MATLAB to fit the phantom data into a calibration curve, which is merged with volunteers' data, to form a reference curve for our specific scanning system.
Results / **Conclusion:** A key feature of our study is to obtain scanner specific calibration curves for non-invasive measurements of absolute human brain temperature. Earlier methods, based on mathematical assumptions, provided a feasible, yet largely unverified approach to register temperature changes. However it is necessary to detect absolute temperature in order to improve our knowledge on temperature distribution in the healthy and injured brain.

Poster Presentation

P07: FABRY pain: understanding pain in FABRY disease

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Background: Fabry disease is a life-limiting genetic metabolic disorder caused by a deficiency of lysosomal alpha-galactosidase A activity. A recent screening study in Austria discovered Fabry associated mutations in 1 out of 4000 births. The mutations lead to progressive accumulation of glycolipids in numerous cell types including neurons. First symptoms include chronic burning pain with attacks of excruciating pain and sensory losses. Enzyme replacement therapy improves the severity of the systemic disease, however is insufficient to treat the persisting pain. Small fiber neuropathy and the accumulation of glycolipids have been linked to pain in Fabry patients.
Methods: Mice deficient of alpha-Galactosidase A ($Gla^{-/0}$) serves as a model of Fabry disease and is used to investigate Fabry related functional changes in nociceptors. Thermal nociception and locomotive performance were monitored from juvenile (w6) to adult (w20) stage in age-matched $Gla^{-/0}$ and wildtype mice. Cultured sensory neurons will be used as a model for nociception in electrophysiological recordings that are specifically designed to reveal functional alterations of $Gla^{-/0}$ vs. wildtype nociceptive neurons. Since deficits in temperature sensation are associated with Fabry pain, the heat-sensitivity of cultured nociceptors was examined.
Results: Hargreaves experiments showed that the onset of Fabry related changes in nociceptors starts around postnatal week 12, evident from the attenuation of heat perception in the hindpaws of $Gla^{-/0}$ mice. However, the $Gla^{-/0}$ mice do not display any motor deficits monitored by RotaRod or quantitative assessment of footfalls and gait. Cultured nociceptors from $Gla^{-/0}$ mice did not reveal any differences in heat sensitivity in electrophysiological recordings. Furthermore, the action potentials from 8-9 and 20-22 week old $Gla^{-/0}$ mice are indistinguishable from action potentials of age-matched controls. However, when $Gla^{-/0}$ nociceptors from 20 week old mice are challenged by a prolonged depolarization, the frequency of action potential firing is significantly increased. Analysis of current-voltage relations of peak-inward currents and sustained outward currents revealed significant changes in $Gla^{-/0}$ nociceptors.
Conclusion: The loss of heat perception in $Gla^{-/0}$ mice contradicts with the unchanged heat-sensitivity of $Gla^{-/0}$ nociceptors in culture, implying a different mechanism for the loss of heat-sensitivity in $Gla^{-/0}$ mice. Further, the increased hyperexcitability of $Gla^{-/0}$ nociceptors from 20 week old mice could be caused by changed ionic conductances observed in these nociceptors. This could be a cause of spontaneous pain attacks observed in Fabry patients, but still needs further investigation.

Poster Presentation

P 08: Identification and characterization of an 'endosomal stress response' pathway

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Background: The accumulation of aberrant proteins leads to severe cellular defects that are frequently associated with cancer and neurodegenerative diseases. Quality control mechanisms exist to detect proteotoxic stress and initiate specific stress responses, some of which are relatively well understood (e.g. unfolded protein response). Little is known how cells react to the accumulation of membrane proteins that cannot be degraded in lysosomes. Normally, these membrane proteins are ubiquitinated and delivered from endosomes to lysosomes via the multivesicular body (MVB) pathway, which is mediated by the endosomal sorting complexes required for transport (ESCRTs). Thus, if the ESCRT machinery fails, membrane proteins accumulate on endosomes. Loss of ESCRT function triggers the correlated regulation of 40 genes on both the mRNA and protein level. Interestingly, these include genes previously associated with stress-responses and protein trafficking. Genetic and cell biological analysis suggests that some could be important downstream effectors of membrane stress.
Methods: We used a genome-wide high-throughput screen on the yeast knockout collection to identify molecular components of the 'endosomal stress response' (ESR).
Results: We have identified the genetic interactome of the MVB pathway, which includes a number of signaling molecules (e.g. kinases, phosphatases, ubiquitin ligases and transcriptions factors). These are prime candidates for mediators on the ESR, whose functional relationship with the MVB pathway we are currently investigating.
Literature:
Schmidt, O., & Teis, D. (2012). The ESCRT machinery. *Curr Biol*, 22(4), R116-20
Tyedmers J., Mogk A., & Bukau B. (2010) Cellular strategies for controlling protein aggregation. *Nat Rev Mol Cell Biol*, 11(11), 777-788.

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Poster Presentation

P 09: A novel lipid sensor in the endosomal membrane

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Background: The endocytic pathway is involved in a large variety of cellular processes such as the uptake of nutrients, the biological response to extracellular stimuli through regulation of recycling or degradation of receptors, and antigen presentation in the immune response. Deficiency in the late endosomal LAMTOR/Ragulator complex leads to a lysosome-related human pathology. However, the precise roles of LAMTOR in endosomal biogenesis are unknown. The LAMTOR complex has a pivotal role in cellular homeostasis, as it has been shown to be essential for MAPK and mTOR signaling from the late endosomal and lysosomal membrane.
Methods / **Results:** We recently obtained several data indicating that LAMTOR has a pivotal role in lipid metabolism, both from unbiased large-scale experiments and from more directed biochemical and cell biological analyses. Firstly, Affymetrix gene chip analysis indicated that LAMTOR regulates lipid synthesis and uptake at the transcriptional level by activating sterol responsive element binding proteins (SREBP) 1 and 2. Secondly, shotgun lipidomics showed that LAMTOR deficient cells have a disturbed lipid content: most strikingly, the neutral storage lipid cholesteryl ester was decreased three-fold. Thirdly, in vitro adipogenesis assays revealed that fat storage capacity was severely disturbed in cultured LAMTOR deficient fibroblasts and macrophages. Fourthly, histological analysis of tissue sections indicated that mice carrying a LAMTOR deficiency specific to adipocytes display severely disturbed cell size and distribution in brown and white adipose tissue.
Conclusion: Based on these results and the available literature, we propose that LAMTOR senses the lipid composition of the late endosomal/lysosomal membrane and regulates mTOR signaling to affect activation of lipid synthesis and uptake by SREBP1 and 2. LAMTOR may also modulate uptake and endocytosis of lipids in an mTOR-independent fashion. We further propose that this causes the observed defects in neutral lipid storage and size and distribution of adipocytes, and contributes to the disturbed endosomal biogenesis observed in LAMTOR2 deficiency patients, and to regulation of cellular proliferation.

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Poster presentation

P 10: Helena, the hidden beauty: resolving the most common West Eurasian mtDNA control region haplotype at the highest resolution by massively parallel sequencing

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Background: The analysis of mitochondrial (mt)DNA is a powerful tool in forensic genetics when nuclear markers fail to give results or maternal relatedness is investigated. The ~1.1 kbp mtDNA control region (CR) contains highly condensed variation and is therefore routinely typed. Some samples exhibit an identical haplotype in this restricted range. Thus, they convey only weak evidence in forensic queries and limited phylogenetic information. However, a CR match does not imply that also the mtDNA coding regions are identical or samples belong to the same phylogenetic lineage. This is especially the case for the most frequent West Eurasian mtDNA CR haplotype 263G 315.1C 16519C, which is observed in various clades within haplogroup H (sometimes referred to as "Helena") and occurs at a frequency of 3-4% in many European populations.
Methods / **Results:** In this study, we investigated the power of massively parallel complete (~16.6 kbp) mtGenome sequencing according to highest forensic standards in - to date - 29 Italian samples displaying the most common West Eurasian CR haplotype - and found an unexpected high diversity. 28 different haplotypes falling into 19 described sub-clades of haplogroup H were revealed in the samples with identical CR sequences.
Conclusion: This study demonstrates the benefit of complete mtGenome sequencing for forensic applications to enforce maximum discrimination, more comprehensive heteroplasmy detection, as well as highest phylogenetic resolution.

Poster Presentation

P 11: Regulation of B cell development and function by long non-coding RNAs

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Background: B lymphocytes, which are a key cell type of the adaptive immune system, are generated in a complex developmental process that is subdivided into several consecutive steps. Progression through the individual stages is tightly controlled by checkpoints, as defects can result in autoimmunity, immunodeficiency or tumorigenesis. The underlying molecular mechanisms that on the one hand allow the establishment of immune function, but on the other hand can contribute to diseases, are not completely understood.
Traditionally, research has focussed on the role of protein-coding genes in the regulation of lymphocyte development and function. However, recent data indicate that the non-coding part of the human genome is extensively transcribed and contributes significantly to the orchestrating and fine-tuning of transcriptional programs both in health and disease. Among the non-coding RNA species identified so far, the group of long non-coding RNAs (lncRNAs), defined by a length of more than 200 nucleotides, is the least understood.
Methods: Here, we have initiated a project that aims to systematically evaluate the role of lncRNA in the B lymphocyte compartment. To find novel lncRNAs, we have isolated distinct B cell stages from the bone marrow (pro-B, pre-BI, pre-BII, immature B) as well as from the spleen (mature B cells, unstimulated or stimulated with anti-CD40L) and have characterized the transcriptome by high throughput sequencing. With Bioinformatics tools, we have identified lncRNAs that are differentially expressed in the respective stages, and have validated these results by qPCR in a larger set of samples. Furthermore, we have used *in vitro* studies to link the differential expression of selected lncRNAs to distinct signalling pathways.
Results: Our preliminary data indicate that B cells express a lot of previously known and novel lncRNAs, but whether they play an important role is unclear. A subset of these lncRNAs is differentially expressed in the distinct developmental stages, suggesting that they may confer a certain function. We have begun to perform *in vitro* perturbation experiments, such as gain-of-function and loss-of-function approaches, to characterize a subset of these lncRNA candidates in more detail.
Conclusion: Our preliminary data suggest that lncRNAs are extensively expressed throughout B cell development, but their functional relevance is still unknown. Based on our RNAseq analysis, we have been able to select a set of promising candidates. We expect that their functional analysis will reveal that they are not transcriptional noise, but rather play an important role in lymphocyte development and function.

Project Overview

S 01: Decision making abilities in patients with multiple sclerosis – Assessment and training

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Background: Patients with multiple sclerosis (MS) have to face important decisions as concerns their medical treatment. Risk understanding and acceptance play a major role. A recent study (Simioni et al., 2012) has found that patients with MS make poor decisions under risk conditions, where explicit numerical information about the options and consequences is given. Further studies are urgently needed to better characterize the mechanisms of decision making in MS and the difficulties the patients encounter in making advantageous decisions. Decisions under risk involve complex numerical competences such as assessing and comparing the probabilities associated with the benefits and risks of a treatment option. For example, a patient is offered a medical treatment that has success in 95% of cases, but it could lead to complications in 5% of cases. It has been shown that patients with cognitive impairments have difficulties to understand and manipulate ratio concepts such as proportions, percentages, and probabilities, and that higher competence in ratio processing predicts more advantageous decisions under risk. It may be therefore expected that increasing an individual's numerical competence (e.g., understanding of ratios) would facilitate advantageous decision making under risk.
Aims: Firstly, this project aims to evaluate the cognitive, emotional, and disease-related factors that may contribute to advantageous decision making in patients with MS *(Part 1: Assessment)*. Secondly, it aims to develop and evaluate targeted intervention methods to improve the patients' competence in decision making and ratio processing *(Part 2: Training)*.
Methods: *Part 1 (Assessment):* Patients with relapse-remitting MS (n = 60) and healthy controls (n = 60) will undergo a comprehensive neuropsychological assessment of numerical competences, understanding of ratio concepts, executive functions, and decision making under risk. *Part 2 (Training):* Patients with MS (n = 40) and controls (n = 40) will first undergo a neuropsychological assessment. Then, they will be randomly assigned to one of two training conditions: a) training on symbolic ratio processing; b) non-numerical training (placebo condition). Training will take ca. 30 min. a day for 5 days. At post-training, participants will be re-evaluated on a selection of neuropsychological tests assessing numerical competences, ratio processing, and decision making. Performance of patients in Part 1 and Part 2 will be compared with that of controls.
Hypotheses: *Part 1 (Assessment):* We expect poorer performance of patients with RRMS relative to controls in executive functions, ratio processing, decision making, and numerical competences that rely on intact fronto-subcortical circuits. Poorer performance of patients with RRMS correlates with longer disease duration, higher relapsing rate, higher disability grade, higher progression index, and poorer health-related quality of life. *Part 2 (Training):* Participants will show improvements following training in the trained domain. We also expect that patients with RRMS will profit most from explicit training on symbolic ratio processing, as they can directly apply the newly learned knowledge in decision making under risk.
Expected outcome: Results will definitely deepen our understanding of the difficulties encountered by patients with MS in daily life decisions. Also, they will give us first insights into how targeted training programmes may help the patients improving their competences with ratio concepts and in making advantageous decisions. Our research will provide professionals with important information for counselling patients during medical treatment.

Project Overview

S 02: Intracranial aneurysm as a hypertensive disease

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Abstract: One main risk factor for the development of intracranial aneurysms may be arterial hypertension. The rupture of an intracranial aneurysm leads to subarachnoid hemorrhage (SAH) from which more than half of the patients die or suffer from disabilities. However, due to methodological causes, the hypothesis that arterial hypertension may be a cause for aneurysm development and its rupture, remains to be proven or disproven. After a SAH patients are sympathetically activated and can suffer from arterial hypertension due to severe pain. Therefore the blood pressure is not evaluable in the acute stage, and a reliable blood pressure history is not always available.

Routine imaging of patients with SAH in order to identify aneurysms include a computed tomography angiography (CTA) and a digital subtraction angiography (DSA). Moreover, in our department every patient with SAH receives a fundoscopy to quantify micro vessel changes, which are related to arterial hypertension. Calcifying macroangiopathy of the supraaortal vessels, as an epiphenomenon of arterial hypertension, can be quantified using CTA, based on a new method, which has been established in Innsbruck.

In addition, there is a known correlation between arterial hypertension and the presence of adrenal adenomas and pheochromocytomas, but a correlation between the incidence of intracranial aneurysms and adrenal tumors has never been investigated.

The aim of the present study is to detect correlations between the presence of intracranial aneurysms and the amount of supraaortal calcification as an epiphenomenon of arterial hypertension. In addition, for the first time, the correlation between intracranial aneurysm rupture and the presence of adrenal tumors as potential causes of secondary hypertension will be investigated.

Project Overview

S 03: Cardiovascular phenotyping of a transgenic mouse model for multiple system atrophy.

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Abstract: Multiple system atrophy (MSA) is a rapidly progressive, fatal neurodegenerative disease presenting with autonomic failure (including prominent cardiovascular dysfunction with neurogenic orthostatic hypotension and nocturnal hypertension), Parkinsonism and cerebellar ataxia in different combinations and severity. The morphological hallmark of MSA are alpha-Synuclein (α-Syn) positive inclusions in the cytoplasm of oligodendrocytes, so-called (oligodendro-) glial cytoplasmic inclusions (GCIs). In the past decade, focus has moved towards characterization of autonomic features that often occur before the onset of motor-symptoms and are therefore also referred to as pre-motor features. These autonomic symptoms involve a broad range including cardiovascular, urogenital, respiratory and sudomotor domains. Over the last decade transgenic MSA models have been developed to provide a testbed for preclinical studies aimed at elucidating the pathogenesis and developing novel therapies. Our work has shown that the PLP-α-Syn mouse model replicates GCIs, MSA like neurodegeneration and motor features similar to Parkinsonism and ataxia occurring in patients. We recently identified autonomic brainstem and spinal cord pathology in PLP-α-Syn mice. Further we showed reduced heart rate variability that is characteristic for the human disease. The focus of the present study is to extend the cardiovascular phenotyping approach by investigating whether abnormalities of blood pressure regulation occur in the PLP-α-Syn mouse model of MSA. Blockers of different contributors to blood pressure control will be evaluated in this project. This study will provide key information regarding the cardiovascular autonomic failure associated with the PLP MSA model and may identify novel targets for intervention.

Project Overview

S 04: microRNAs in axonal regeneration: regulation of mir-138 and mir-21 by gp130 signaling in peripheral nerve injury and recovery

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Abstract: Nerve injuries cause severe disability and strongly compromise the quality of life. Recovery and functional outcome after nerve injury is yet insufficient and at present, no therapy can overcome the limitations in posttraumatic peripheral nerve repair. A better understanding of the cellular and molecular mechanisms promoting nerve regeneration would help in developing new therapeutic strategies.

Changes in gene expression and signaling after neuronal damage can be induced by factors released from non-neuronal cells. Local inflammatory events at the lesion site attract immune cells and this is accompanied by the elevated levels of neurotropic cytokines, such as IL-6, a well-accepted neuron survival and regeneration factor. For signal transduction the receptor beta subunit 130 (gp130) heterodimerizes with the IL-6 binding receptor alpha subunit. Using SNS-gp130 conditional knockout mice where functional gp130 is selectively deleted from peripheral sensory neurons, we have previously shown that gp130 significantly contributes to complex interactions between immune cells and nerves. Moreover, we have recently found that gp130 is crucial for the regeneration of peripheral sensory neurons *in vivo* and *in vitro*.

Axon regeneration involves several important signaling pathways. IL-6 binding to ILR/gp130 was demonstrated to stimulate axon regeneration by transducing signaling through JAK1 and JAK2 which consequently activates transcriptional factor STAT3 and MAP kinase pathways. Another important regulator of peripheral axon regeneration, PTEN, functions as endogenous regeneration suppressor.

The discovery of microRNAs (miRNAs) has changed our understanding of post-transcriptional regulation of gene expression and added complicity to the hypothesis of the signal cascade activation and compartmentalization. miRNAs were shown to be crucial for the development and function of nervous system. Growing evidence supports the hypothesis that peripheral nerve regeneration may also be regulated by miRNAs. Among others, mir-21 and mir-138 were shown to be expressed in neurons and to be differently regulated after nerve injuries. Moreover, in cancer and myeloma cells, IL-6/STAT3 and PTEN/Akt pathways were recently demonstrated to be regulated by mir-21 and mir-138. How this regulation may function in peripheral axon injury and regeneration remains obscure.

In our preliminary experiments we identified expression of mir-21 and mir-138 in dorsal root ganglion (DRG) explants obtained from SNS-gp130-/- and control mice. Moreover, our unpublished data suggest that mir-21 and mir-138 are differently regulated in SNS-gp130-/- vs control mice after spared nerve injury. In the proposed project we want to confirm and further evaluate biological significance of mir-21 or mir-138 deregulation after axon injury and during regeneration in SNS-gp130-/- mice. Further we will focus on studying of validated miRNA target genes that are involved in IL-6/STAT3/JAK/PTEN pathway.

Project Overview

S 05: Tryptophan and kynurenine metabolism in alcohol dependent patients in acute and medium term withdrawal

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Abstract: 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 underlying the 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) is up to now not established.

This study aims to investigate the dynamics of tryptophan metabolism and kynurenine catabolism in alcohol dependent patients during acute and medium-term alcohol withdrawal. Serum concentrations of tryptophan, kynurenine and its catabolites will be measured repeatedly during four weeks of acute and medium term withdrawal in order (1) to establish long-term patterns of tryptophan and kynurenine metabolism and (2) to investigate their association with clinical symptoms during the different stages of alcohol withdrawal.

New insights in the mechanisms of recovery from alcoholism may lead to improvement of therapeutic and rehabilitation schemes for this disease.

Project Overview

S 06: Identification and assessment of altered miRNA expression profiles to improve early prostate cancer detection

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Abstract: Prostate cancer (PCa) is the most commonly diagnosed malignancy and the second leading cause of cancer-related mortality in men. In recent years much effort has been made to identify and study novel biomarkers like prostate cancer antigen 3 (PCA3) and the fusion gene TMPRSS2:ERG to improve PCa detection but to date, only the biomarker prostate specific antigen (PSA) is routinely used by urologists. However, despite its tremendous value in clinical practice, PSA is not an ideal biomarker. Thus, identification of new molecular biomarkers is urgently required in order to improve an early PCa detection and management. In this context, micro-RNAs (miRNAs) and their potential use as diagnostic and- or prognostic biomarkers have become focus of investigation for many malignancies. miRNAs are important regulators of mRNA and protein expression. Given their ability to target mRNAs with imperfect complementarity, miRNAs are considered to be very important gene regulators and dysfunction in miRNA expression can have severe consequences. miRNA expression profiling could already identify and distinguish tumor sub types in different malignancies. Therefore, the applicant want to clarify within this project, whether deregulated expression profiles of specific miRNAs can be used to discriminate healthy from organ confined and metastatic PCa patients with the aim to establish a basis for the development of a novel diagnostic miRNA biomarker panel to improve early PCa detection.

Project Overview

S 07: Peroxisomal import pathways and their role in *A. fumigatus* virulence and adaptation

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Abstract: Fungal infections constitute an important issue for modern medicine, as fungi infect particularly immunocompromised patients. Aspergillosis has increased considerably over the last years making it the most common invasive fungal infection besides candidiasis. Infections with *Aspergillus fumigatus* have become the leading cause of death in persons with bone marrow transplants, cystic fibrosis, HIV or tuberculosis. As treatment of fungal diseases is limited, new strategies are urgently required.

Peroxisomes are single membrane organelles that compartmentalize a wide range of metabolic functions in eukaryotic cells. Due to the diversity of metabolic pathways in peroxisomes, their content varies depending on the species, cell or tissue type, as well as on environmental conditions. Peroxisomal matrix proteins are imported from the cytoplasm into the peroxisomes through peroxisomal targeting signal 1 (PTS1) or the less common peroxisomal targeting signal 2 (PTS2). PTS1 import is dependent on its soluble receptor Pex5. PTS2 import depends on Pex7 and furthermore on the fungal specific co-receptor Pex20. It has been described for fungal plant pathogens that blocking either one of the two peroxisomal import pathways turned the fungi avirulent or at least led to attenuated virulence. As Pex20 is fungal specific and is not present in human, it might offer a putative target for antifungal treatment. However, the role of Pex5 and Pex20 in human fungal pathogens is still unclear. Therefore, the objective of the proposed project is to study the biological role of these two receptors, Pex5 and Pex20, in relation to peroxisomal import pathways and their possible impact on *A. fumigatus* virulence and adaptation to nutrient limitation and stresses.

Project Overview

S 08: The role and impact of the tumor endothelial regulator "Robo4" in prostate cancer

Isabel Heidegger

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Abstract: Communication between tumor cells and vascular endothelial cells and tumor-induced angiogenesis is a hallmark of cancer. The roundabouts are transmembrane receptors expressed in developing tissues, which regulate the structure, motility and migration of various subsets of vascular cells and cells interacting with the blood vessel wall. High expression of roundabouts is found in the central nervous system (Robo1, Robo2, Robo3) as well as in the neovascular endothelium (Robo4). In generally, Robo4 functions as an endothelial specific guidance receptor at sites of active angiogenesis, in particular tumor angiogenesis. It has been identified as a tumor endothelial marker in genome-wide analysis of the tumor endothelium. The ligands of Robo4, the Slit proteins were found to contribute to tumor angiogenesis by interacting with VEGF signaling thereby leading to vessel stabilization. In prostate cancer, which is the most common cancer in men, the role of Robo4 has not been investigated yet. We hypothesize that Robo4 together with its ligands plays a crucial role in prostate tumor neoangiogenesis and aim to characterize is function with regard to anti-angiogenic therapy. Using in vitro (cell culture co-culture systems) and in vivo (Chorioallantoic membrane assay) prostate cancer models and modulating Robo4/Slit expression we will analyze the impact on tumor growth, colony formation, cell migration, cell invasion, tube formation and vessel stabilization. Moreover we will evaluate and characterize Robo4/Silt expression on patients´ tumor and non-malignant tissue. Finally we plan to correlate Robo4 expression in tumor samples with clinical parameters like tumor grade, prognostic factors, progression free survival and overall survival.

Project Overview

S 09: Formaldehyde metabolism – On the role of formaldehyde in inflammation

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Abstract: Formaldehyde (HCHO) is the simplest aldehyde that shows high reactivity towards cellular molecules. Formaldehyde is ubiquitously present as environmental pollutant associated with a broad range of harmful effects ranging from irritations, lung function impairment, neurotoxicity to cancer development.

Of note, formaldehyde is constantly produced by endogenous metabolic routes. Estimated formaldehyde concentrations in human blood are in the range of 10-100 µM. Several biochemical pathways use formaldehyde and formate as essential intermediates, thereby folate-mediated one carbon metabolism being of central importance. Recent data show that formaldehyde levels are increased in patients suffering from different types of cancers, and also cancer cells and cell lines in culture are able to release formaldehyde. Since now, the role of endogenous formaldehyde formation under inflammatory conditions has not been investigated.

We aim to investigate the formaldehyde metabolism in human peripheral mononuclear cells (PBMC) and in A549 lung adenocarcinoma cells after stimulation with inflammatory molecules. High performance liquid chromatography (HPLC) will be employed to detect formaldehyde and other aldehydes in cells and culture supernatants and transcriptional rates of enzymes involved in formaldehyde metabolism will be analyzed. Additionally, homocysteine concentrations will be measured, as the methionine homocysteine pathway is a potential sensitive target of increased formaldehyde. The results from the proposed experiments are expected to further strengthen the knowledge on the endogenous formaldehyde production and its role in inflammation.

Project Overview

S 10: GC production in the thymus and its influence on T cell development

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Abstract: Glucocorticoids (GC) are a class of steroid hormones which are part of the feedback mechanism in the immune system, turning down inflammatory processes by binding to their receptor, the GR. Recent evidence indicates that GC enables the selection of T cells in the thymus that express T cell receptors (TCR) with sufficient affinity for self-peptides that subsequently build the peripheral T cell repertoire. Indeed, T-cells of a T cell specific GR-deficient mouse failed to respond to specific antigens and viral infection. Mechanistically, GC are thought to inhibit TCR signaling of those thymocytes that would otherwise be negatively selected. This directly suggests a crucial role of GC in shaping the TCR repertoire.

It has been thought for a long time that GC were exclusively synthesized by the adrenal gland. At present, there is a large body of evidence showing the presence of extra-adrenal synthesis of GC in the thymus that may play an important role in T cell development and selection. Controversial is the localization of GC production in the thymus: while some reports clearly show that thymic epithelial cells (TEC) are the only cellular source in the thymus capable of synthesizing GC, others claim that thymocytes are the main source of GC production in the thymus.

Since GC play an important role in T cell development and it is not clear whether thymocytes are able to produce GC, this work proposal aims to address this. We will assess the expression and activity of enzymes needed to synthesize GC, such as CYP11A1 and CYP11B1, at different stages of T cell development. Moreover, expression and activity of 11□HSD1, an enzyme capable of converting inactive precursors into active GC, will be monitored. To that end, different thymocyte subsets will be sorted and assessed for enzyme activity in an *in vitro* thymocyte differentiation model (OP9-DL1 serum-free co-culture system). In this model thymocyte differentiation and function can be manipulated independent of thymic epithelial cells and in the absence of serum-derived confounding compounds. Production of GC will be determined in culture supernatants by ELISA. To assess 11□HSD1 activity, precursor molecules will be added at several stages of thymocyte differentiation and the generation of GC determined. In addition, GC will be exogenously added to monitor their effects on thymocyte development. We will also perform experiments with GR-/- thymocytes from $GR^{lck\text{-}Cre}$ mice to determine whether any endogenously produced GC affects T cell development and whether the effects are indeed mediated via the GR.

In summary, the proposal aims to elucidate whether thymocytes produce GC and affect their own development. If this is the case it would strongly support the concept that (thymus-derived) GC shape the T cell repertoire and are directly required for immune fitness. Moreover, new opportunities may be opened not only to increase the efficacy of GC treatment but also to dampen GC-dependent side effects during the treatment of autoimmune diseases.

Project Overview

S 11: Caspase-2 in cell death induced by polyploidization

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Abstract: Members of the caspase (cystein-dependent aspartate-specific protease) family are considered critical regulators of two major biological processes, i.e. apoptotic cell death (caspase 3, 6, 7, 8, 9, 10) and induction of inflammation (caspase 1, 4, 5, 8). Despite two decades of research the function of Caspase-2, the most conserved member of the family, remains poorly defined. On the one hand, the epistatic position attributed to Caspase-2 within the apoptotic signalling varies greatly depending on the cell death paradigm and experimental system being considered. On the other hand, our knowledge on caspase-2 substrates remains scarce, contributing to the enigmatic trait of this protease.

According to a structural classification, Caspase-2 is grouped among the initiating caspases, as it possesses a long N-terminal pro-domain that can serve to bind scaffold proteins able to induce autoproteolytic caspase activation. Most notably, Caspase-2 can be activated when engaged via its pro-domain in a trimolecular complex with PIDD and RAIDD, named PIDDosome. The only cell death paradigm in which PIDDosome appears to be a crucial inducer of apoptosis is the so called "Chk1-suppressed pathway" (Ando et al, 2012; Sidi et al, 2008), that postulates that PIDDosome assembly requires the induction of DNA damage via ionizing radiation in conjunction with the inhibition of the checkpoint kinase-1 (Chk-1).

With the aim to better define the cues leading to Caspase-2 activation in this paradigm, we hypothesized that Chk-1 might not exert a direct inhibition on PIDDosome assembly but rather that this latter event might be induced by the deregulation of the cell cycle induced by the combination treatment. To this end, we noted that the induction of mitotic failure and therefore polyploidization by various means triggered Caspase-2 activation and subsequent induction of cell death, independently of Chk-1 inhibition and direct induction of DNA damage.

Here I propose a research project aimed to define i) the molecular similarities and differences between the Chk1-suppressed pathway and cell death induced by polyploidization, ii) the exact epistatic relationship between Caspase-2 and other apoptotic regulators in this pathway and iii) the general validity of our finding when tested in different experimental systems. Somewhat independently of the above aims, I plan to exploit the prominent role that Caspase-2 has in the death triggered by polyploidization for the realization of a proteomics based screen aimed to identify Caspase-2 substrates on a global scale.

Successful completion of this project will allow better defining the molecular cues leading to Caspase-2 activation and the relationship with other more understood cell death pathways. The systematic identification of Caspase-2 cellular substrates will answer the long standing question on whether Caspase-2 possesses a set of unique substrates or rather shares the same targets with effector caspases.

Project Overview

S 12: AN4022–A novel HDAC complex component as basis for a novel antifungal therapy

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Abstract: Filamentous fungi have enormous impact on mankind in both positive and deleterious aspects, ranging from producers of pharmaceutical drugs to life threatening pathogens. Like all eukaryotic, fungi have to org anize large amounts of DNA in the cell nucleus. This is achieved by the formation of chromatin, an elaborate and highly dynamic structure composed of DNA, histones and other proteins. Since accessibility of the genetic material by enzyme complexes involved in DNA-dependent processes such as replication, transcription, recombination, or repair has to be stringently controlled, chromatin offers another level of regulation in addition to sequence specific transcription factors. Fungi, especially in a pathogenic setting, have to adapt quickly in response to environmental changes, *e.g.* the host defense. Therefore, regulation of chromatin structure and thus gene regulation have to be highly dynamic. Post-translational modification of core histones, *e.g.* reversible acetylation catalyzed by histone acetyltransferases and histone deacetylases (HDACs), is an important factor in mediating this flexibility. We could demonstrate that RpdA, a class 1 HDAC and ortholog of yeast Rpd3, in contrast to *Saccharomyces cerevisiae,* is essential for growth and development of the model ascomycete *Aspergillus nidulans* and the human pathogen *Aspergillus fumigatus*, indicating an important role for virulence. Sequence alignments revealed the presence of a C-terminal extension in filamentous fungal Rpd3-type HDACs, harboring a highly conserved sequence motif, which is not present in yeast or mammalian orthologs and is crucial for the function of fungal enzymes. Taken together, these features make these enzymes promising targets for the development of specific antifungal compounds, in particular as they exert their function by the interaction with other proteins. However, a precise understanding of RpdA-type HDACs and their structural peculiarities is a prerequisite to achieve this goal. Therefore, we started to assess, if the composition of RpdA complexes reflects their special status by the presence of additional specific partner proteins. Indeed, affinity purification combined with LC-MS/MS analysis identified a previously uncharacterized conserved fungal protein (AN4022) in addition to already known Rpd3 interacting partners from yeast and other organisms. Importantly, physical interaction of AN4022 with RpdA could be confirmed by *vice versa* purifications. Orthologs of AN4022 can only be found within the group of *Eurotiomycetales*, including a number of important fungal pathogens like *A. fumigatus, A. terreus, A. flavus, Penicillium marneffei, Coccidioides immitis,* or *Histoplasma capsulatum*. This makes AN4022 an ideal candidate to study functions of RpdA complexes specific for this group of filamentous fungi. To unravel the biological role of AN4022 and to establish its crucial role in formation, stabilization, and proper function of fungal-specific HDAC complexes is the objective of the proposed project.