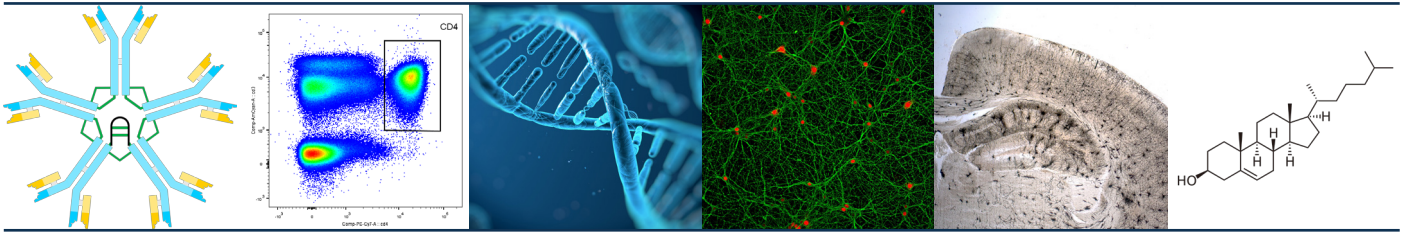
LIFE SCIENCE INNSBRUCK PhD SYMPOSIUM 2016

MEDIZINISCHE UNIVERSITÄT INNSBRUCK

universität innsbruck



1st Life Science Innsbruck PhD Symposium
31st March - 1st April 2016

Pictures:
Institute for Neuroscience
Selma Tuzlak
www.wikipedia.com
www.wallpaperup.com

Edited by: Marco Grasse
Layout style from: „Evolution Across Fields“ Abstract book by Mandy Quade, Nina Schleimer and Andreas Wessel
Also referenced: 4th SPP1399 Abstract book by Marco Grasse, Thorsten Reusch, Hinrich Schulenburg

Contents

PROGRAMME

10:00 - 11:30 PhD Student's Career Workshop (with plenary speakers)
M.01.470/490

11:30 - 12:30 *Lunch break*

12:30 - 12:45 Welcome
M.EG.180

12:45 - 13:30 Plenary lecture, chair: Anupam Sah
M.EG.180
John Cryan, University College Cork, Ireland - Neuroscience
A gut feeling about the brain: How microbes could modify our minds

13:45 - 14:45 Short talks *parallel sessions*

Chair:	Claudia Schmuckmair	**Chair:**	Veronika Klepsch & Wilfried Posch
13:45 - 14:00 M.EG.180	Heike Mrowetz (neuroscience) Ageing Influences the Response of Hippocampal Progenitor Cells to Demyelination	**13:45 - 14:00** M.EG.200	Katia Schöler (cell biology) Apoptosis via the miR-17~92-Bim axis in Development and Cancer
14:00 - 14:15 M.EG.180	Michael Unger (neuroscience) Early changes in adult hippocampal neurogenesis in transgenic mouse models for Alzheimer's disease	**14:00 - 14:15** M.EG.200	Florian Handle (cell biology) SOCS3 influences AR activity and stem cell-associated genes during anti-androgen treatment in prostate cancer
14:15 - 14:30 M.EG.180	Kathrin Kähler (neuroscience) The Neuroretina in Multiple System Atrophy: Morphological and Functional Aspects in Plp-α-SYN Mice	**14:15 - 14:30** M.EG.200	Daniela Ortner (immunology) Cancer immunosurveillance by natural killer cells depends on signals derived from Langerhans cells upon carcinogen application
14:30 - 14:45 M.EG.180	Carlo Bavassano (neuroscience) Generation and Characterization of a Human Neuronal Model of *Spinocerebellar Ataxia* Type 6 via Induced Pluripotent Stem Cell Differentiation	**14:30 - 14:45** M.EG.200	Natasa Prokopi (immunology) Immune evasion by melanoma: Modification of the skin and lymph node immune cell network in a spontaneous melanoma mouse model

14:45 - 16:45 Joint poster session #1
Aula / Foyer

Please be at your poster if your abstract number is:
even 14:45-15:45
odd 15:45-16:45

Cell Biology Abstract-numbers: 01-16
Immunology Abstract-numbers: 32-42
Neuroscience Abstract-numbers: 53-70

16:45 - 17:45 Short talks *parallel sessions*

Chair:	Bruno Benedetti & Petronel Tuluc	**Chair:**	Ulrike Binder & Zoltan Banki
16:45 - 17:00 M.EG.180	Vincenzo Mastrolia (cell biology) Importance of the calcium channel α2δ-1 subunit for insulin release and glucose homeostasis	**16:45 - 17:00** M.EG.200	Marion Steger (immunology) Complement-Opsonization of *A. fumigatus* Modifies Dendritic Cell Function
17:00 - 17:15 M.EG.180	Marta Campiglio (cell biology) The PKC C1 domain of STAC3 critically determines its isoform-specific interactions with L-type calcium channels in skeletal muscle triads	**17:00 - 17:15** M.EG.200	Rita Carramalho (immunology) Mitochondrial genes as novel diagnostic markers for mucormycoses
17:15 - 17:30 M.EG.180	Johannes Burtscher (neuroscience) Oxidative phosphorylation in the healthy and in the epileptic mouse brain	**17:15 - 17:30** M.EG.200	Parul Chandorkar (immunology) Establishment Of 3D Human Lung Tissue Model To Study Polymicrobial Infections
17:30 - 17:45 M.EG.180	Isabella Cera (neuroscience) Structure-Function analysis of neuronal Satb2-protein complexes	**17:30 - 17:45** M.EG.200	Luca Pangrazzi (immunology) Oxidative stress & age related impairments in the maintenance of immunological memory

18:00 - 18:45 Plenary lecture, chair: Dorothea Orth-Höller
M.EG.180
Stefan Niemann, Research Centre Borstel, Germany - immunology
Whole genome sequencing for analyzing *M. tuberculosis* transmission, resistance and evolution

18:45 *Cracker, cheese & wine at the posters*
Aula / Foyer

Programme

10:00 - 10:45 M.EG.180
Plenary lecture, chair: Teodor Yordanov
Thomas Kirchhausen, Harvard University, USA - Molecular Cell Biology
Cellular dynamics imaged in real time and in 3D using a lattice light sheet microscope

11:00 - 12:00 Short talks *parallel sessions*

	Chair: Julia Höfer & Simon Spränger	**Chair:** Edith Sturm & Igor Theurl
11:00 - 11:15 M.EG.180 / M.EG.200	Simona Migliano (cell biology) The role of Vps4 on endosomes during the last seconds of intraluminal vesicle biogenesis	S. zur Nedden (neuroscience) Role of protein kinase C-related kinase 1 (PKN1) in neuroprotection
11:15 - 11:30 M.EG.180 / M.EG.200	Valentina Sladky (cell biology) Dissecting the role of caspase-2 in "mitotic catastrophe"	Serena Quarta (neuroscience) Sphingosine-1-phosphate and its receptor S1P3 induced neurite retraction via Rho/ROCK
11:30 - 11:45 M.EG.180 / M.EG.200	Dulce Lima Cuna (cell biology) Induced pluripotent stem cell technology for disease modelling of rare genodermatoses – iPSC-derived keratinocytes from patients with congenital ichthyosis	Birgit Waldner (cell biology) Molecular Determinants of Serine Protease Specificity
11:45 - 12:00 M.EG.180 / M.EG.200	Ruth Röck (cell biology) Impact of kinase activating and inactivating patient mutations on binary PKA interactions	Carles Urbiola (immunology) LCMV-GP Pseudotyped Oncolytic Vesicular Stomatitis Virus for the Treatment of Prostate Cancer

12:00 - 13:00 *Lunch break*

Programme

13:00 - 15:00 Aula / Foyer
Joint poster session #2

Please be at your poster if your abstract number is:
even 13:00-14:00
odd 14:00-15:00

Cell Biology Abstract-numbers: 17-31
Immunology Abstract-numbers: 43-52
Neuroscience * Abstract-numbers: 71-89
* and remaining categories
Abstract-numbers: 88 & 89

15:00 - 17:00 Award ceremony

15:00 - 15:45 M.EG.180/200
MCBO best paper award
Natascha Hermann-Kleiter, **Victoria Klepsch** et al. (2015): The Nuclear Orphan Receptor NR2F6 is a Central Checkpoint for Cancer Immune Surveillance

Alexandra Pinggera et al. (2015): CACNA1D De Novo Mutations in Autism Spectrum Disorders Activate Cav1.3 L-Type Calcium Channels

SPIN best paper award
Luca Zangrandi et al. (2016):The G-protein biased partial kappa opioid receptor agonist 6' GNTI blocks hippocampal paroxysmal discharges without inducing aversion

15:45 - 16:30 M.EG.180/200
MCBO Alumni talk - M. Haffner
Prostate Cancer Genomes: Complexity at Multiple Levels

SPIN Alumni talk - M. Ramberger
$CD4^+$ T cell reactivity to orexin/hypocretin in patients with narcolepsy type 1

16:30 - 17:00 Short talks / Poster prices

17:00- 17:45 M.EG.180/200
Annual lecture, chair: Christine Bandtlow
Jürgen Knoblich, Institute of Molecular Biotechnology, Vienna, Austria
Growing human brains in a dish: 3D organoid culture as a model for human brain development and disease

18:00 Aula / Foyer
Buffet & grand finale (party)

• Lunch is not provided by the organisers - a small supermarket is directly next to the CCB

• Poster - your poster will stay at your assigned posterwall the entire symposium time. Please remove it immediately after the last talk on friday. Please be at your poster at the indicated time (programme):

even posternumbers at the first hour of your assigned postersession
odd posternumbers at the second hour of your assigned postersession

Ageing Influences the Response of Hippocampal Progenitor Cells to Demyelination

Heike Mrowetz[1,2], Simona Lange[1,2], Francisco J. Rivera[1,2], Ludwig Aigner[1,2], Barbara Klein[1,2]

[1]Institute of Molecular Regenerative Medicine, Paracelsus Medical University, Salzburg, Austria
[2]Spinal Cord Injury and Tissue Regeneration Center Salzburg, Salzburg, Austria

heike.mrowetz@pmu.ac.at

Background: In addition to neurogenesis, the adult hippocampus provides a pool of different types of progenitor cells, which may contribute to regenerative processes. Apart from DCX+ neuronal progenitor cells (NPCs), also oligodendrocyte precursor cells (OPCs) are present in the hippocampus, which can differentiate to remyelinating oligodendrocytes. As aging restricts regeneration, we investigated how hippocampal NPCs and OPCs contribute to the generation of new neurons and oligodendrocytes upon demyelination and whether ageing influences this process.
Methods: Cuprizone-induced demyelination and subsequent remyelination were studied in 2-months-old and 10-months-old male C57BL/6J mice. The control group received a normal diet for 8 weeks. The treatment groups received food pellets pre-mixed with 0.2% cuprizone for 6 weeks to induce demyelination. Afterwards, depending on the group, a normal diet was resumed for 1 or 2 weeks for remyelination. To analyze proliferation and cell survival, mice were injected 6 times with bromodeoxyuridine (BrdU) at the onset of remyelination.
Results: In the hippocampus, treatment with cuprizone induced an efficient demyelination followed by remyelination, which was assessed by MBP staining. During remyelination the number of proliferating NPCs (PCNA+DCX+) changed slightly only in young animals. To evaluate the generation of mature neurons (NeuN+), the number of NeuN+BrdU+ cells was analyzed, but no changes were detected. The proliferation of cells of the oligodendrocyte lineage (Olig2+PCNA+) was only significantly increased early after remyelination started exclusively in young animals. During remyelination, the percentage of Olig2+ cells BrdU+ differentiated oligodendrocytes were detected.
Discussion: Proliferation of NPCs and Olig2+ cells was slightly changed only in young animals, whereas the number of BrdU+ mature neurons was unchanged in either age group, possibly suggesting a different role for young NPCs. During remyelination the percentage of Olig2+ cells which were also CC1+ increased in middle-aged animals, which suggests that middle-aged animals might be more effective in generating differentiated oligodendrocytes. However, in middle-aged animals no BrdU+ differentiated oligodendrocyte (Olig2+CC1+BrdU+) cells were detected within two weeks of remyelination, suggesting that the differentiation takes longer in middle-aged animals compared to young animals.

Early changes in adult hippocampal neurogenesis in transgenic mouse models for Alzheimer's disease

Aims: In this study we examined adult hippocampal neurogenesis at different time points of Alzheimer's disease (AD) progression in two AD mouse models.
Methods: Immunohistochemical fluorescence staining (IHC) was performed on free floating brain slices of the transgenic APP Swedish PS1 ΔE9 and Tg2576 mouse model. The rate of neurogenesis, i.e. the number of proliferating (PCNA+) and neuronal precursor cells (DCX+) was quantitatively analysed by confocal laser scanning microscopy (LSM 710). For analysis of cell survival APP Swedish PS1 ΔE9 animals were injected with bromodeoxyuridine (BrdU) for 5 consecutive days four weeks before perfusion and cell survival (BrdU+) and the number of newly formed neurons (BrdU+/NeuN+) was quantified.
Results: Surprisingly, first changes in adult hippocampal neurogenesis were already detected in 3 months old AD mice compared to wildtype (WT) animals prior to amyloid-beta plaque formation. At this early stage of AD the number of proliferating cells and the number of proliferating neuronal precursor cells were significantly increased in the AD mice compared to WT. Analysing BrdU+ cells and newly formed neurons (BrdU+/NeuN+) revealed significantly decreased numbers of cell survival and new neurons in AD animals compared to WT.
Conclusion: Our results suggest that first alterations in hippocampal neurogenesis take place before amyloid-beta plaque formation and we assume that hyperproliferating neuronal progenitor cells have a reduced potential to differentiate into new neurons at early stages of AD and therefore contribute to the cognitive decline in AD pathology.

Michael Unger[1,2], J. Marschallinger[1,2], J. Kaindl[1,2], S. Rossner[3], M.T. Heneka[4], A. Van der Linden[5], L. Aigner[1,2]

[1]Institute of Molecular Regenerative Medicine, Paracelsus Medical University, Salzburg, Austria
[2]Spinal Cord Injury and Tissue Regeneration Center Salzburg (SCI-TReCS), Salzburg, Austria
[3]Paul Flechsig Institute for Brain Research, University of Leipzig, Leipzig, Germany
[4]Clinical Neuroscience, Department of Neurology, University of Bonn, Bonn, Germany
[5]Department of Biomedical Sciences, University of Antwerp, Antwerp, Belgium

michael.unger@pmu.ac.at

The Neuroretina in Multiple System Atrophy: Morphological and Functional Aspects in Plp-a-SYN Mice

Kathrin Kaehler, Hartwig Seitter, Bettina Tschugg, Edith Sturm, Adolf M. Sandbichler, Nadia Stefanova, Alexandra Koschak

Dopaminergic neurons in the central nervous system exhibit physiological abnormalities and dysfunction in neurodegenerative diseases like Parkinson's disease and Multiple System Atrophy (MSA). These abnormalities can result from exceeding aggregation of α-synuclein (α- SYN). Due to reported morphological and functional changes of the retinal structure in MSA patients, we aimed at investigating the retinal morphology in homozygous transgenic (PLP)-α-SYN mice overexpressing human α-SYN under the proteolipid protein (PLP)-promoter. Immunohistochemical analyses on vertical retinal sections were performed to investigate retinal dopaminergic neurons, the tyrosin-hydroxylase (TH) positive amacrine cells, of transgenic and wild type (WT) animals of two different age groups (two months, one year). In PLP-α-SYN animals, numerous TH-positive processes appeared to reach into deeper strata of the inner plexiform layer (IPL), and cell bodies were deformed. Distinct α-SYN signal occurred in different retinal cell layers of PLP-α-SYN mice, but not in WT mice. Published data proved the activity of the PLP oligodendrocyte promotor only in optic nerve but not in retinal tissue. We therefore aim to investigate the origin of α-SYN in PLP-α-SYN retinae. Further immunohistochemical experiments will also focus on retinal glia cells due to the glial pathology of MSA. Multielectrode array analyses will elicit whether changes in the contrast sensitivity function in PLP-α-SYN mice are comparable to human deficits. Our data implicate an impairment of retinal neurons in this MSA model, which may also underlie visual deficits reported in MSA patients.

kathrin.kaehler@uibk.ac.at

Generation and Characterization of a Human Neuronal Model of Spinocerebellar Ataxia Type 6 via Induced Pluripotent Stem Cell Differentiation

Carlo Bavassano[1], A. Eigentler[1], R. Stanika[2], S. Boesch[3], R. Nat[1], G. Dechant[1]

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

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 CaV2.1, which, in the brain, is particularly highly expressed in the cerebellum. SCA6 mutation consists of a short expansion of a polyglutamine stretch located in the cytoplasmic C-terminal tail of the 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-CaV2.1 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).
Control and SCA6 patient-derived iPSC lines were generated using Yamanaka´s reprogramming factors. Neuronal differentiation was achieved following a milestone-based protocol, and each stage of differentiation was defined by morphological criteria and the expression of stage-specific markers. In addition, a genetically encoded fluorescent reporter was used for the identification of mature differentiated neurons. The resulting SCA6 patient-derived neurons expressed the typical proteins of differentiated neurons, including cytoskeletal proteins (Tau, MAP2, beta tubulin III), synaptic proteins (PSD95, Synapsin, Synaptohphysin), voltage-gated ion channels (KV1.1, NaV1.1, NaV1.2), neurotransmitter-related enzymes and transporters (GAD67, vGlut1, vGlut2). Patch clamp recordings revealed the capability of firing action potentials and eliciting voltage-dependent calcium currents, and CaV2.1 channel protein was expressed both in control and SCA6 neurons. The characterization of this human model will include the analysis of CaV2.1 currents, synaptic transmission, CaV2.1 channel protein subcellular distribution 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 on CaV2.1 channel protein functionality in human neurons.

carlo.bavassano@i-med.ac.at

Apoptosis via the miR-17~92-Bim axis in Development and Cancer

Katia Schöler[1§], V. Labi[1§], F. Klironomos[2], S. Peng[3], T. Chakraborty[4], M. Munschauer[2], M. Landthaler[2], N. Rajewsky[2], K. Rajewsky[2], A. Villunger[1]

[1]Division of Developmental Immunology, Medical University Innsbruck, Austria
[2]Max Delbrück Center for Molecular Medicine, Berlin, Germany
[3]IDMO Beijing, China
[4]ModeRNA Therapeutics, Cambridge, Massachusetts, USA
[§] authors contributed equally

katia.schoeler@i-med.ac.at

MicroRNAs modulate gene expression by interacting with messenger RNAs. Small size and imperfect target recognition empowers a given microRNA to reduce the levels of virtually hundreds of proteins. Thus, identification of direct versus indirect and the functionally relevant targets remains a major challenge. MicroRNAs of the miR-17~92 cluster have attracted attention due to their critical involvement in embryogenesis and frequent overexpression in cancer. One of the top predicted combinatorial targets of miR-17~92 is the pro-apoptotic Bcl-2 protein Bim. Generating an in vivo murine system of conditional mutagenesis of all miR-17~92 binding sites in the Bim 3'UTR we uncovered for the first time the true relevance of direct miR-17~92-Bim interactions in normal physiology.
Homozygous germ-line Bim 3'UTR mutated mice die perinatally hence identifying miR-17~92-Bim interactions as an essential regulatory mechanisms in embryogenesis. Lymphoproliferation in Bim+/- mice can be prevented by replacement of the wild type with the Bim 3'UTR mutated allele. Unexpectedly, B progenitor fitness is not impaired in homozygous B-cell specific Bim 3'UTR mutated mice. These results indicate cell-type/context-dependence of functional miR-17~92-Bim interactions. Using our unique mouse model, we aim to further investigate a causal link between Myc, miR-17~92 and Bim in B-cell lymphomas, as extensively proposed in literature.

SOCS3 influences AR activity and stem cell-associated genes during anti-androgen treatment in prostate cancer

Florian Handle[1], Holger H.H. Erb[1,2], Birgit Luef[1], Julia Hoefer[1], Dimo Dietrich[3] Walther Parson[4,5], Glen Kristiansen[3], Frédéric R. Santer[1*], Zoran Culig[1*]

[1]Division of Experimental Urology, Department of Urology, Medical University of Innsbruck, Innsbruck, Austria
[2]Yorkshire Cancer Research Unit, University of York, York, United Kingdom.
[3]Institute of Pathology, University Hospital Bonn, Bonn, Germany
[4]Institute of Legal Medicine, Medical University of Innsbruck, Innsbruck, Austria
[5]Forensic Science Program, The Pennsylvania State University, University Park, Pennsylvania, USA
*Joint senior authors

florian.handle@i-med.ac.at

The pro-inflammatory cytokine interleukin 6 (IL6) is associated with bad prognosis in prostate cancer (PCa) and implicated in progression to castration resistance. Suppressor of cytokine signaling 3 (SOCS3) is an IL6 induced negative feedback regulator of the IL6/Janus Kinase (JAK)/signal transducer and activator of transcription 3 (STAT3) pathway. In this study we could show that the SOCS3 promoter is hypermethylated in cancerous regions compared to adjacent benign tissue by using methylation-specific qPCR. Methylation was significantly increased with Gleason score and tumor stage. To study the functional impact of low SOCS3 expression during anti-androgen treatment we carried out in vitro experiments. By performing lentivirus-mediated knock-down experiments, we could demonstrate for the first time that SOCS3 regulates IL6/JAK/STAT3 signaling in AR positive LNCaP cells. Additionally, we could show that SOCS3 mRNA is up-regulated by the anti-androgens bicalutamide and enzalutamide. This effect is mediated via altered STAT3 signaling and can be blocked by the STAT3 inhibitor gallielalactone. After three weeks of enzalutamide treatment, knock-down of SOCS3 led to enhanced AR activity in an inflammatory setting. Furthermore, the stemness/self-renewal associated genes SOX2 and NANOG were strongly up-regulated by the long-term treatment and modulation of SOCS3 expression was sufficient to counteract this effect. These findings prove that SOCS3 plays an important role in prostate cancer during anti-androgen treatment in an inflammatory setting.

CANCER IMMUNOSURVEILLANCE BY NATURAL KILLER CELLS DEPENDS ON SIGNALS DERIVED FROM LANGERHANS CELLS UPON CARCINOGEN APPLICATION

Daniela Ortner-Tobider[1], Christoph H. Tripp[1], Sandrine Dubrac[1], David E. Schlögl[1], Kerstin Komenda[1], Björn E. Clausen[2], Patrizia Stoitzner[1]

[1]Department of Dermatology, Venereology and Allergology, Innsbruck Medical University, Innsbruck, Austria
[2]Institute for Molecular Medicine, University Medical Center of the Johannes Gutenberg-University Mainz, Mainz, Germany

daniela.ortner-tobider@i-med.ac.at

In this project we wanted to understand the innate immune mechanisms occuring during chemical tumor initiation with the carcinogen 7,12-dimethylbenz(a)anthracene (DMBA). Langerin-positive dermal DC and Langerhans cells (LC) proved to be important at the early stage of carcinogenesis because tumor formation was accelerated in mice depleted of Langerin-positive DC/LC just at timepoint of DMBA-mediated tumor induction. In accordance with these findings we detected an accumulation of DNA-damged keratinocytes and enhanced expression of NKG2D ligands in skin devoid of Langerin-positive DC/LC. After DMBA application more NK cells can be found in the epidermis and in vivo depletion of NK cells accelerated tumor development in a similiar way than the absence of LC. Besides, epidermis devoid of LC showed a significanly lower level of TNFα-mRNA expression upon chemical treatment. In vivo blockade of TNFα at the timepoint of DMBA application led to more DNA damage in keratinocytes. Our findings demonstrate an important link between LC and NK cells during cancer immunosurveillance of chemically treated skin.

IMMUNE EVASION BY MELANOMA: MODIFICATION OF THE SKIN AND LYMPH NODE IMMUNE CELL NETWORK IN A SPONTANEOUS MELANOMA MOUSE MODEL

Melanoma is the most fatal type of skin cancer. Despite the knowledge acquired so far regarding the etiology of melanoma, there is still a lot of progress to be made in order to elucidate the basic immune evasion mechanisms. Skin dendritic cells (DC) are the first defense line of the immune system when it comes to skin-related diseases. Moreover, they function as a bridge between innate and adaptive immunity. However, little is known regarding the function of the different skin DC subsets and their interactions with T cells in a tumor setting. Ectopic expression of the neuronal receptor, metabotropic glutamate receptor 1 (Grm1), in melanocytes has been described to be involved in melanoma development in humans. The overexpression of Grm1 in melanocytes of the transgenic tg(Grm1)EPv mouse strain leads to spontaneous melanoma development. In this study we characterized the alterations that take place in the skin and in the draining lymph nodes regarding the different skin DC subsets as well as CD4+ and CD8+ T cells. Our results show that tumor development modifies the skin immune cell network. Understanding the processes underlying these changes will offer useful information in regards to the role of the different skin DC subsets in tumor antigen presentation and activation of CD8+ T cells. This knowledge will provide the basis that will allow us to enhance the efficacy of existing immunotherapies against melanoma.

Natasa Prokopi[1], D. G. Mairhofer[1], C. H. Tripp[1], D. Ortner[1], K. Knapp[1], K. Komenda[1], S. Chen[2], B. E. Clausen[3], P. Stoitzner[1]

[1]Department of Dermatology, Venereology and Allergology, Innsbruck Medical University, Innsbruck, Austria
[2]Susan Lehman Cullman Laboratory for Cancer Research, Rutgers University, Piscataway, New Jersey, USA
[3]Institute for Molecular Medicine, University Medical Center of the Johannes Gutenberg University, Mainz, Germany

anastasia.prokopi@i-med.ac.at

Importance of the calcium channel α2δ-1 subunit for insulin release and glucose homeostasis

Vincenzo Mastrolia[1], S. M. Flucher[1], G. J. Obermair[1], M. Drach[2], E. Renström[3], A. Schwartz[4], J. Striessnig[5], B. E. Flucher[1], P. Tuluc[1,5]

[1]Department of Physiology and Medical Physics, Medical University Innsbruck, Austria
[2]Department of General Pathology, Medical University Innsbruck, Austria
[3]Department of Clinical Sciences Malmö, Sweden
[4]College of Medicine, University of Cincinnati, USA
[5]Pharmacology and Toxicology, Institute of Pharmacy, University of Innsbruck, Austria

vincenzo.mastrolia@i-med.ac.at

Reduced pancreatic β-cell function or mass is the critical problem in the development of diabetes. β-cell insulin vesicle release depends on the calcium influx through high voltage-gated calcium channels (HVCC). Calcium influx also contributes to β-cell electrical activity, insulin granule priming, and insulin synthesis. The α2δ-1 subunit is a key regulator of HVCC membrane incorporation and function. Here we show that genetic deletion of α2δ-1 in mice results in glucose intolerance and the development of severe diabetes due to decreased insulin release while insulin sensitivity remains unaffected. The stronger basal insulin release in female mice alleviates the diabetes symptoms in most α2δ-1-/- females while in all males and some females the disease phenotype escalates in a reduction of β-cell mass. Together these findings demonstrate that loss of calcium channel α2δ-1 subunit function increases the diabetes susceptibility in a gender dependent manner.

The PKC C1 domain of STAC3 critically determines its isoform-specific interactions with L-type calcium channels in skeletal muscle triads

Skeletal muscle excitation-contraction coupling critically depends on the physical interaction between the voltage-gated calcium channel CaV1.1 (DHPR), acting as voltage sensor, and the SR calcium release channel (RyR1). The skeletal muscle-specific DHPR components essential for EC coupling are the CaV1.1 II-III loop and the auxiliary β1a subunit. Recently, STAC3 (src homology 3 and cysteine rich domain 3) has been identified as an additional essential EC coupling protein. Further investigations indicated that STAC3 functions in membrane trafficking of CaV1.1, and linked STAC3 to a rare muscle disease Native American Myopathy (NAM). Interestingly, STAC proteins form a family of three, and while STAC3 expression is restricted to skeletal muscle, STAC1 and STAC2 are expressed in brain and a variety of other tissues, but their subcellular localizations or functions are not known. These findings raise the questions as to whether STAC3 binds exclusively to the skeletal muscle DHPR, whether its interaction with the EC coupling complex is stable or dynamic, which domain is involved in the interaction with the DHPR and whether other STAC proteins can associate to and modulate voltage-gated calcium channels. Here, we reconstituted dysgenic (CaV1.1-null) myotubes with different STAC and CaV1 subunits and applied colocalization assays and fluorescence recovery after photobleaching (FRAP) to demonstrate that: (1) STAC3 forms stable complexes with the skeletal muscle DHPR. (2) The NAM mutation does not abolish or diminish the stability of the interaction with CaV1.1. (3) The cardiac/neuronal CaV1.2 very efficiently recruits STAC3 to skeletal muscle DHPR complexes and the two heterologous protein partners associate with each other as stably as the native partners STAC3 and CaV1.1. (4) STAC3 is the unique partner of the skeletal muscle DHPR. (5) Both STAC1 and STAC2 associate with CaV1.2 at different extents and possibly modulate voltage-gated calcium channels in their native tissues. Using STAC2/STAC3 chimeras and site-directed mutagenesis, we could demonstrate that (6) the PKC-C1 domain of STAC3 is crucial for its interaction with L-type calcium channels and that (7) two critical amino acids, the side chains of which are exposed in a pocket in the tertiary structure of the PKC C1 domain, are involved in the association with the DHPR. Ongoing electrophysiology will reveal whether mutation of these two amino acids results in impairs membrane incorporation of functional CaV1.1 channels.

Marta Campiglio, Bernhard E. Flucher

Department of Physiology and Medical Physics, Medical University Innsbruck, Austria

marta.campiglio@i-med.ac.at

Oxidative phosphorylation in the healthy and in the epileptic mouse brain

Johannes Burtscher[1], Luca Zangrandi[1], Christoph Schwarzer[1], Erich Gnaiger[2,3]

[1]Dept. Pharmacology, Medical University Innsbruck, Peter-Mayr-Str. 1a, 6020 Innsbruck, Austria
[2]D. Swarowski Research Laboratory, Department of Visceral, Transplant and Thoracic Surgery, Medical University Innsbruck, Anichstr. 35, 6020 Innsbruck, Austria
[3]OROBOROS INSTRUMENTS, Schöpfstr. 18, 6020 Innsbruck, Austria

johannes.burtscher@i-med.ac.at

Mitochondrial dysfunction appears to be a common factor in neurodegenerative diseases. Strikingly, neurodegenerative diseases show regional specificity in vulnerability and follow distinct patterns of neuronal loss. A challenge is to understand, how mitochondrial failure in particular brain regions contributes to specific pathological conditions.
High-resolution respirometry revealed significant differences of Complex I- and II- (CI and CII) linked oxidative phosphorylation (OXPHOS) capacity and coupling control between motor cortex, striatum, hippocampus and pons of naïve mice. CI-linked respiration was highest in motor cortex. In contrast, CII-linked capacity was especially important in the striatum. Apparent excess capacities of the electron transfer system (ETS) over OXPHOS also differed between regions. These differences may indicate risk factors for region-specific neuronal vulnerabilities.
In the kainic acid (KA) model of temporal lobe epilepsy in mice, we observed markedly decreased absolute CI- and CII- linked oxygen consumption and also decreased ETS-capacity in the injected dorsal hippocampus 2 days after KA. When normalized to ETS-capacity, CII-linked respiration was significantly increased compared to controls. 3 weeks after KA-injection, absolute CII-linked oxygen consumption reached control levels, but was still elevated when normalized to ETS-capacity. Absolute CI-linked oxygen consumption and ETS-capacity were still decreased.
In summary, respirometric OXPHOS analysis allows detailed analysis of mitochondrial function from small amounts of specific tissues (about 2 mg). It thus enables comparison of different brain tissues implicated in neurodegenerative diseases of the healthy mouse and disease models, while leaving enough material for further studies on the tissues.

Structure-Function analysis of neuronal Satb2-protein complexes

Increasing evidence obtained in a variety of non-neuronal cell types indicates that higher-order chromatin arrangements and dynamic interactions of interphase chromosomes with protein complexes at the inner nuclear membrane play essential roles in the activation or silencing of genes. In neurons such mechanisms have not yet been investigated.
The chromatin organizer protein “special AT-rich sequence binding protein 2” (Satb2) is a regulator of chromatin architecture since it can induce formation of chromosomal loops, thereby ensuring coordinated regulation of multiple close and distant genes.
In order to identify Satb2 interactome in CNS, we performed a co-immunoprecipation of Satb2 from mouse neonatal and adult cortical lysates followed by mass-spectrometry analysis. Besides already described Satb2 interactors (NurD Complex), additional proteins were identified as novel binding partners of Satb2. Among them were a group of RNA binding proteins, proteins of the nuclear pore complex, as well as proteins that belong to the inner nuclear membrane. These results reveal potentially novel aspects of Satb2 functions: Satb2 might be part of a protein complex which mediates interactions of specific chromatin loops with the inner nuclear membrane in neurons, thereby affecting transcriptional activity of specific loci. Additional we found that Satb2 knockout mice have deficits in long term memory formation suggesting that chromatin reorganization of neurons mediated by Satb2 could be correlated with cognitive functions in vivo.

Isabella Cera, Andreas Abentung, Clemens Jaitner, Chethan Reddy, Patrick Feurle, Galina Apostolova, Georg Dechant

Institute for Neuroscience, Medical University Innsbruck

isabella.cera@i-med.ac.at

Complement-Opsonization of *A. fumigatus* Modifies Dendritic Cell Function

Marion Steger[1], W. Posch[1], C. Lass-Flörl[1], H. Haas[2], D.Wilflingseder[1]

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

marion.steger@i-med.ac.at

Background: Interactions of dendritic cells (DCs) with complement-opsonized and non-opsonized Aspergillus fumigatus strains and various mutants thereof were investigated. The opsonization pattern of the different strains and mutants, the binding and internalization by dendritic cells as well as the cytokine secretion and initial signaling pathways were investigated.
Methods: Fungi were opsonized using normal human serum as complement source. The opsonization pattern, binding of conidia to DCs and internalization were characterized by FACS analyses. Inhibition of fungal growth in presence of DCs and interactions with complement receptors were detected using confocal microscopy. Furthermore, phosphorylation of ERK1/2 and p38 were detected by immunoblot analysis.
Results: We could demonstrate in this study that melanin and β-1,3-glucan have high impact on the fungal virulence compared to the wildtype Aspergillus strains. With respect to dendritic cell binding and internalization complement-opsonization of conidia enhanced these processes compared to their non-opsonized counterparts independent on the fungal strain used.
Conclusion: These data revealed, that melanin and β-1,3- glucan are key effectors of masking complement deposition and binding of conidia by DCs. However opsonization of swollen conidia enhanced internalization in DCs as well as their maturation and production of pro-inflammatory cytokines, thereby resulting in a favorable immune response. These in vitro studies propose that the use of immune cells, like DCs or neutrophils, in combination with complement opsonins might act as potent vaccines against invasive aspergillosis.

Mitochondrial genes as novel diagnostic markers for mucormycoses

Background: Mucormycoses are highly destructive infections which are challenging in their diagnoses. Difficulties in setting the diagnoses are due to: i) unspecific signs and symptoms, which are easily confused with other invasive fungal infections; ii) mechanically delicate hyphae which easily break and leak out; and iii) low pathogen loads compared with bacterial or viral infections. Hence, the aim of this study was to select novel, easily amplifiable molecular markers particularly located in robust cell organelles (e.g., mitochondria). Moreover, these markers are able to differentiate between colonization and infection as amount of mitochondria varies between active and resting stages.
Methods: A strain collection of 131 Mucorales isolates were identified to species level by ITS (internal transcribed spacer region) sequencing. Two mitochondrial genes [NADH dehydrogenase subunit 2 (nad2) and the large subunit ribosomal RNA (rnl)] were evaluated for their discriminative power on species and genus level of mucormycetes. Single Nucleotide Polymorphisms (SNPs) were identified, catalogued and evaluated for their discriminatory power. For the normalisation of mitochondrial ratio, three housekeeping genes were added to the gene set. The ratio between mitochondrial and nucleic genes will potentially allow us to distinguish between resting conidia and proliferating hyphae.
Results: Mitochondrial markers and housekeeping genes were amplified for the five major clinically relevant mucormycetes. Sequence analysis revealed 30 SNPs for nad2 and 22 SNPs for rnl. Both mitochondrial markers had sufficient discriminatory power to distinguish between four out of five species; the sibling species Lichtheimia corymbifera and Lichtheimia ramosa could not be discriminated. A total of three regions in the nad2 marker of respectively, 18, 10 and 50 nucleotides long were found. The first two regions shared six SNPs each, while in the third, eight polymorphic sites were observed in total. The described regions are in silico suitable for High Resolution Melt Analysis.
Conclusion: Both mitochondrial genes deliver good resolution power which makes them attractive for the design of a diagnostic tool.

Rita Carramalho[1], T. Larentis[1,2], C. Lass-Flörl[1], M. Lackner[1]

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

rita.ferreira@i-med.ac.at

Establishment Of 3D Human Lung Tissue Model To Study Polymicrobial Infections

Parul Chandorkar[1], W. Posch[1], M. Steger[1], M. Blatzer[1], C. Ammann[2], M. Hermann[3], C-Lass-Flörl[1], D. Wilflingseder[1]

[1]Division of Hygiene & Medical Microbiology, Medical University Innsbruck, Austria
[2]Department of Experimental Orthopaedics, Medical University Innsbruck, Austria
[3]Department of Anaesthesiology & Critical Care Medicine, Medical University Innsbruck, Austria

parul.chandorkar@student.i-med.ac.at

Background: Aspergillus fumigatus is a saprophytic fungus which causes clinical syndromes ranging from colonization to deep-seated infections. Infection mainly begins in the lung tissue of individuals with a challenged immune system. Current studies primarily involve the use of animal models and cell lines to understand the fungal invasion process. However, it is essential to unfold mechanisms underlying the primary interactions and invasion process between the respiratory epithelium and the pathogen. Multiple predisposing factors such as role of immune cells, or cytokines released at the site of infection need to be studied additionally. Therefore there is need for a sophisticated in vitro lung model.
Methods: We employed Normal human bronchial epithelial (NHBE) cells in air-liquid interface culture (ALI) to set up the 3D lung model and further studied their development using immunofluorescence and live cell microscopy. We compared differentiated NHBE cells with ´ready-to-use´ respiratory epithelial cells (Epithelix). Preliminary interactions of *A.fumigatus* with MucilAir™ cells were studied using Scanning electron microscopy (SEM).
Results: Analysis over time by confocal microscopy showed that NHBE cells differentiated in ALI to form tight junctions, produced mucus and developed cilia. These findings were comparable with MucilAir™ airway cells. SEM studies of MucilAirTM infected with Aspergillus demonstrated that the host cell surface produced factors that inhibited the fungus from producing hyphae.
Conclusion: Our preliminary data will have future implications to use differentiated NHBE cells in combination with immune cells in a 3D setting reflecting in vivo conditions. This model will support better understanding of pathogenesis, detection, and treatment of polymicrobial infections. It may also have a broad impact in dissecting immune responses during co-infections and could additionally represent a valid alternative method to animal experimentation.

Oxidative stress & age related impairments in the maintenance of immunilogical memory

Luca Pangrazzi, Andreas Meryk, Beatrix Grubeck-Loebenstein

Division of Immunology, Research Institute for Biomedical Aging Research, University of Innsbruck

luca.pangrazzi@uibk.ac.at

Aging induces a basal level of inflammation throughout the body, a condition known as inflammaging, which contributes to immunosenescence. New strategies to counteract immunosenescence in the elderly are needed, in particular by improving the maintenance of immunological memory. It has been demonstrated that memory T cells and long-lived plasma cells home to bone marrow niches, well organized structures which promote the survival of these cells through homeostatic proliferation. $CD4^+$ and $CD8^+$ effector memory T cell survival is promoted by IL-7 and IL-15. IL-7 is believed to be important for long-lived memory T cells while IL-15 is mostly important for short-lived $CD28^-$ senescent T cells, of which accumulation is associated with mortality in old age. The expression of effector memory T cells and proinflammatory factors were investigated in bone marrow mononuclear cells (BMMC) using qPCR and FACS, finding that, with age, IL-7 decreases while IL-15, IL-6, TNFα, IFNγ and IL1β increase. Stimulation of peripheral blood mononuclear cells (PBMC) with IFNγ and TNFα lead to increased IL-15 expression in $CD11c^{++}$ cells and monocytes, well-known producers of this cytokine. A correlation was found between ROS levels and IL-15 expression in the same cell populations. Incubation of stimulated PBMCs with ROS scavengers N-acetylcysteine and vitamin C completely neutralized the effects of proinflammatory molecules in $CD11c^{++}$ cells and monocytes. This indicates that oxidative stress may contribute to the age-related impairments in the maintenance of immunological memory. Antioxidant treatment may be an important strategy to counteract immunosenescence, reducing the level of proinflammatory cytokines in old age.

The role of Vps4 on endosomes during the last seconds of intraluminal vesicle biogenesis

Simona M. Migliano, M.A. Y Adell, S. Upadhyayula, Y. Bykov, S. Sprenger, M. Pakdel, G.F. Vogel, G. Tzu-Yung Jih, M. Babst, M. Hess, J. Briggs, T. Kirchhausen, D. Teis

The endosomal sorting complex required for transport (ESCRT) mediates topological unique membrane deformation and scission reactions essential during the biogenesis of multivesicular bodies (MVBs) and other cellular processes. Among these are the abscission at the end of cytokinesis, plasma membrane repair, release of retroviral particles from host cells and nuclear envelope reformation.
A common characteristic in all these processes is the typical spiralling assembly of ESCRT-III proteins and the recruitment of the AAA ATPase Vps4. Together ESCRT-III and Vps4 are mediating the last steps of membrane deformation and scission.
However, the recruitment, assembly and disassembly dynamics of these protein complexes on endosomal membranes are not yet understood.
By using a novel Vps4-GFP fusion protein, endogenously expressed in the yeast model S. cerevisiae, we were for the first time able to follow the dynamics of Vps4 on endosomes, in vivo and in real time. We addressed this by 3D lattice light sheet microscopy (with close to single molecule sensitivity) and correlative light- and electron microscopy, to monitor the ultrastructural distribution of Vps4-GFP on endosomes.
Completing these datasets with biochemical and chemical genetic approaches, we were able to follow the cumulative recruitment of Vps4-GFP to ESCRT-III on single moving endosomes in vivo. For the first time we observed distinct Vps4-GFP assembly events (30-80 molecules) lasting for 10-30 seconds before the catastrophic dissociation of the protein complexes.
All together these approaches allow us to get an insight in the last seconds of the intraluminal vesicle formation and MVB biogenesis and how this may correlate with membrane deformation and vesicle release.

simona.migliano@i-med.ac.at

Dissecting the role of caspase-2 in "mitotic catastrophe"

Caspase-2, the most conserved member of the caspase family, has been implicated in several physiological processes ranging from metabolism to DNA damage responses and maintenance of genome integrity. Despite these implications, the role of caspase-2 is still controversial and little is known yet about its substrates or cellular events triggering caspase-2 activation. Moreover, murine models deficient in either caspase-2 or members of its postulated activation platform, a multi-protein complex termed PIDDosome, do not reveal phenotypes expected for any of its proposed functions, highlighting the fact that the role of caspase-2 is still poorly defined.
As caspase-2 has been proposed to be involved in DNA repair and maintenance of genome integrity, here we aim to elucidate the role of caspase-2 in "mitotic catastrophe", a term frequently abused to describe different types of cellular responses including different types of cell death as well as senescence in cells undergoing defective mitosis. In order to shed light on its role in cell death upon "mitotic catastrophe", genetic as well as pharmacologic tools were employed to define triggers activating selectively caspase-2. This approach led to the identification of a set of cell cycle-related cues that can activate caspase-2 upon defective mitosis in a PIDDosome dependent manner resulting either in senescence or cell death.

Valentina Sladky, Fabian Schuler, Luca Fava, Andreas Villunger

Division of Developmental Immunology, Medical University of Innsbruck

valentina.sladky@i-med.ac.at

Induced pluripotent stem cell technology for disease modelling of rare genodermatoses – iPSC-derived keratinocytes from patients with congenital ichthyosis

Dulce Lima Cunha[1,2], K.M. Eckl[1,5], M. Rauch[1], R. Casper[2], M. Gupta[3], M. Schmuth[4], J. Zschocke[1], T. Saric[3], H.C.Hennies[1,2,5]

[1]Div. of Human Genetics, Medical Univ. of Innsbruck, Austria
[2]Cologne Center for Genomics, Center for Dermatogenetics, Univ. of Cologne, Germany
[3]Inst. for Neurophysiology, Univ. Hospital, Cologne, Germany
[4]Dept. of Dermatology, Medical Univ. of Innsbruck, Austria
[5]Dept. of Biological Sciences, University of Huddersfield, UK

dulce.de-lima@i-med.ac.at

Autosomal recessive congenital ichthyosis (ARCI) is a rare skin disorder mainly characterized by impaired function of the skin barrier, leading to generalized scaling of the skin and erythema. It is a very heterogeneous disorder, with mutations in 9 genes currently known to be associated with ARCI and comprising 3 major clinical forms of the disease, also with different course and severity. Treatments for this disorder are only symptomatic, leading to the urgent need for suitable disease models not only to study the disease mechanisms but also to develop new treatments.
The generation of induced pluripotent stem cells (iPSC) from adult somatic cells opened a new field of research with focus on the use of these cells for therapeutic applications. Because iPSC carry the same genetic information as the adult cells used for reprogramming, these cells are a highly valuable tool for patient-specific disease modelling.
Our goal is to establish an iPSC-based in vitro skin model system in order to develop and test new therapies for ARCI and other congenital skin diseases. We have generated several iPSC lines using a non-integrating lentiviral system from skin fibroblasts of both healthy donors and distinct ARCI patients with different causal mutations: one patient with a missense mutation in TGM1 and a second patient with a nonsense mutation in PNPLA1. A third patient-specific iPSC line was generated from a patient with trichothiodystrophy, a congenital disorder that, among other signs, includes an ichthyosis phenotype.
All iPSC lines were thoroughly characterized to validate their pluripotency and to confirm that no genetic modifications occurred during reprogramming. The resulting iPSC share common features with ESC, like morphology, expression of pluripotency markers, and the ability to differentiate into cells from the three germ layers.
No chromosomal aberrations or DNA modifications were detected by karyotype and STR analysis. Analysis of whole genome expression pointed to a clear pluripotency profile of all iPSC lines. Global DNA methylation is currently being analysed to assess the active status of pluripotency gene promoters. Importantly, all ARCI iPSC lines present the same causal mutations as the respective parental fibroblasts, confirming their patient-specific status.
iPSC differentiation into ectodermal fate and then human basal keratinocytes (iPSC-hK) showed that, after approx. 25 days, iPSC-hK present high expression of basal keratinocytes-specific markers KRT5 and KRT14, and no pluripotent markers expression is detected after 7 days into the differentiation process. At early stages (day 7-14), ectodermal markers p63 and K18 are present, but we can detect a decrease in their expression by day 25, corresponding to what happens in human epidermis development. At this stage, clear similarities can be seen in the morphology between the iPSC-derived keratinocytes and human primary keratinocytes. This cell population is now being further characterized and used for generation of 3D full skin models in order to mimic the ARCI skin phenotype. Our approach thus promises personalized cell models for the study of ARCI as well as for development of advanced therapeutic interventions.

Impact of kinase activating and inactivating patient mutations on binary PKA interactions

The second messenger molecule cAMP links extracellular signals to intracellular responses. The main cellular cAMP effector is the compartmentalized protein kinase A (PKA). Upon receptor initiated cAMP-mobilization, PKA regulatory subunits (R) bind cAMP thereby triggering dissociation and activation of bound PKA catalytic subunits (PKAc). Mutations in PKAc or RIa subunits manipulate PKA dynamics and activities which contribute to carcinogenesis, hormone excess or hormone deficiency and resistance. Here we extended the application spectrum of a Protein-fragment Complementation Assay based on the Renilla Luciferase to determine binary protein:protein interactions (PPIs) of the PKA network. We compared time- and dose-dependent influences of cAMP-elevation on mutually exclusive PPIs of PKAc with the phosphotransferase inhibiting RIIb and RIa subunits and the protein kinase inhibitor peptide (PKI). We analyzed PKA dynamics following integration of patient mutations into PKAc and RIa. We observed that oncogenic modifications of PKAc(L206R) and RIa(Δ184-236) as well as rare disease mutations in RIa(R368X) affect complex formation of PKA and its responsiveness to cAMP elevation. With the cell-based PKA PPI reporter platform we precisely quantified the mechanistic details how inhibitory PKA interactions and defined patient mutations contribute to PKA functions.

Ruth Röck, Johanna Mayrhofer, Verena Bachmann, Eduard Stefan

ruth.roeck@uibk.ac.at

Role of protein kinase C-related kinase 1 (PKN1) in neuroprotection

Stephanie zur Nedden[1], Thomas Gruber[2], Haelewyn Benoit[3], Cyrille Orset[3], Gottfried Baier[2], Gabriele Baier-Bitterlich[1]

[1]Medical University of Innsbruck, CCB Biocenter/Neurobiochemistry, Innsbruck/Austria
[2]Department for Pharmacology and Genetics, Division of Translational Cell Genetics, Innsbruck, Austria
[3]Experimental Stroke Research Platform- Bd H. Becquerel, Caen/France

stephanie.zur-nedden@i-med.ac.at

Current treatments for ischemic stroke patients aim to restore vascular function, but up to date no therapeutic approaches that promote endogenous neuroprotection and regeneration have been approved. Cerebral ischemia triggers the activation of multiple signaling cascades that regulate the critical balance between cell death and survival. In this study we aimed to investigate the role of protein kinase C-related kinase 1 (PKN1), a member of the protein kinase C superfamily, in the pathology of Ischemia/Reperfusion (I/R) injury. PKN1, the predominantly expressed PKN family member in the central nervous system, has been implicated in cancer, apoptosis and cytoskeletal regulation, but little is known about its function in brain. We have tested PKN1 knockout (KO, from Peter Parker/Francis Crick Research Institute, UK) and Wildtype (WT) mice in an in vivo middle cerebral artery occlusion (MCAo) stroke model and an in vitro I/R model. Our preliminary data suggest that in the absence of PKN1 both, the in vivo lesion volume is significantly smaller and functional recovery is significantly enhanced after MCAo in mice. Concomitantly, we observed an augmented phosphorylation and activity of the critical survival kinase AKT/PKB and suppressed apoptosis (as measured by caspase-3 activation) in postischemic neuronal cerebellar granule cells (Cgc) from PKN1-/- mice. This suggests that PKN1 (patho)-physiologically counteracts the signaling pathway leading to AKT/PKB-mediated neuroprotection. Compared to WT Cgc, ischemic Cgc from PKN1 KO mice also showed a higher activation of the growth and plasticity-associated protein-43 (GAP-43), an important player in axonal regeneration. Accordingly, PKN1 KO Cgc have a significantly enhanced axonal outgrowth capacity on both, growth permissive Laminin and chondroitin sulfate proteoglycans (CSPG), inhibitory substrates which are found in the glial scar, and are responsible for the growth-limiting extracellular environment after stroke. This effect is diminished upon reintroduction of human PKN1, but not altered by the kinase dead version, showing that the growth inhibiting influence of PKN1 depends on its kinase activity. Taken together we provide strong genetic and biochemical evidence suggesting that the absence of PKN1, a protein kinase predominantly expressed in neurons, boosts axonal growth and survival of ischemic neurons in vitro and reduces I/R pathology in the stroke-relevant in vivo model of MCAo. Targeting both, cell survival and axonal regeneration by only one substrate is highly preferable and therefore makes PKN1 an interesting candidate for neuroprotective therapeutic interventions.

Sphingosine-1-phosphate and its receptor S1P3 induced neurite retraction via Rho/ROCK

Serena Quarta[1], Maria Camprubí-Robles[1], Rüdiger Schweigreiter[2], Christine E. Bandtlow[2], Theresa Martha[1], Antonio V. Ferrer-Montiel[3], Michaela Kress[1]

[1]Div. Physiology, DPMP, Medical University Innsbruck, Austria
[2]Div. Neurobiochemistry, Biocenter, Medical University Innsbruck, Austria
[3]Instituto de Biologia Molecular y Celular, Universitas Miguel Hernández, Alicante, Spain

serena.quarta@i-med.ac.at

Aims: The bioactive lipid sphingosine 1-phosphate (S1P) via binding to metabotropic G protein-coupled receptors (GPCR) can activate the small GTPases Rho and Rac which have been associated with neuroregeneration. We explored the importance of S1P and S1P GPCRs for neurite outgrowth of peripheral sensory neurons.
Methods: Mouse adult dorsal root ganglion (DRG) neurons cultures (24 h) were used for in vitro experiments. Experimental sciatic nerve crush injury was followed by sensory and motor behavioral testing in mice with a global deletion of S1P3 (S1P3-/-).
Results: DRG neurons exposed to S1P presented reduced outgrowth capacity in vitro. Moreover, S1P induced retraction of neurites in a dose- and time-dependent manner. S1P was observed to activate RhoA in a pull-down assay and the in vitro S1P induced retraction was reversed by C3-toxin, a specific Rho inhibitor, or the ROCK inhibitor Y2763. The S1P1 agonist SEW2871 treatment neither caused signs of neurite retraction nor activation of RhoA in a pull-down assay. In contrast, neurons from S1P3-/- mice showed a significantly increased total neurite length after 1 µM S1P. In line with these results, thermal sensitivity recovered faster after nerve crush injury in S1P3-/- mice compared to wt animals in vivo. Preliminary evidences from gait analysis (Catwalk® system) indicated signs of improved motor recovery in S1P3-/- mice.
Conclusions: Our findings indicate that S1P can act as regulator of neuronal outgrowth in vitro and of peripheral nerve regeneration in vivo. S1P3 receptor appears to mediate neurite retraction via Rho and ROCK activation.

Molecular Determinants of Serine Protease Specificity

Birgit J. Waldner[1], Julian E. Fuchs[1], Roland G. Huber[1,2], Susanne von Grafenstein[1], Michael Schauperl[1], Christian Kramer[1], Klaus R. Liedl[1]

[1]Institute of General, Inorganic and Theoretical Chemistry, University of Innsbruck, Innsbruck, Austria
[2]Bioinformatics Institute (BII), Agency of Science, Technology and Research (A* STAR), Singapore

birgit.waldner@uibk.ac.at

Despite their often very similar folds, members of the same protease family show different substrate specificity, depending on the physiological processes they are part of. This difference in specificity is quantitatively reflected in the so-called cleavage entropy, a sub-pocket-wise and overall specificity score based on cleavage data from the MEROPS database.
For serine proteases there have been many attempts to explain specificity on the structural basis of particular systems, however to our knowledge there is no approach so far that allows prediction of protease specificity based on intrinsic characteristics of serine proteases.
To understand the mechanism of protease recognition in biological environment, investigation of the interactions at protein-protein interfaces is of crucial importance. As for snake venom metalloproteases it has been shown that specificity can be rationalized through backbone flexibility without taking into account any enthalpic components, we studied the impact of binding site flexibility on specificity in selected serine proteases with Trypsin-like fold. In addition, we investigated the impact of enthalpic factors on substrate recognition using the program GRID to get a complete view on the factors that drive substrate specificity in serine protease sub-pockets.
First, we want to present a quantitative correlation between the local dynamics at the binding site of a series of homologous serine proteases with Trypsin-like fold obtained from molecular dynamics simulations and the specificity of the investigated proteases. Second, we want to present enthalpic contributions to serine protease substrate recognition that are considered through determination of local interaction potentials of selected probes at protease sub-pockets using GRID on snapshots taken from the molecular dynamics simulations. Through a combination of sub-pocket-wise thermodynamic and flexibility data, we can rationalize specificity of the investigated serine proteases and discuss the theory of conformational selection in protease substrate recognition and the application of our findings in drug design methods.

LCMV-GP Pseudotyped Oncolytic Vesicular Stomatitis Virus for the Treatment of Prostate Cancer

Carles R. Urbiola[1], Janine Kimpel[1], Frédéric Santer[2], Zoran Culig[2], Guido Wollmann[1], Dorothee Holmvon Laer[1]

[1]Department of Virologie, Medical University Innsbruck
[2]Urology Clinic, Medical University of Innsbruck

carles.urbiola@i-med.ac.at

Prostate cancer (PCa) is the second leading cause of cancer death in the U.S. and Europe. Diagnosed at early stages, prostate cancer can be surgically removed. However, despite many research efforts, long-term effective therapies are not available. Oncolytic viruses (OV) that preferentially replicate in and kill tumour cells are a potent novel treatment option for cancer patients after failure of common therapeutic strategies such as chemotherapy or surgery. Through cell lysis, OV set free tumour antigens, which in combination with the OV adjuvant effect, unleashes a strong anti-tumour immune response. Our group previously reported that oncolytic Vesicular Stomatitis Virus (VSV) pseudotyped with the LCMV glycoprotein (VSV-GP) is a promising, highly efficient and safe oncolytic virus. Here, we propose the use of the oncolytic VSV-GP for the treatment of prostate cancer.
We used prostate cancer cell lines and primary cultures from patient samples to test the efficacy of VSV-GP in prostate cancer. We analysed oncolytic efficiency as well as the role of the innate immune response in therapy outcome. VSV-GP was further tested in vivo both in a xengoraft and a syngeneic mouse model. In addition, IFN-I response was modulated either using the Jak1/2 inhibitor, Ruxolitinib (Novartis), or by knocking down the IFNAR1 gene in the tumour.
VSV-GP exhibited high oncolytic efficiency in vitro, efficiently killing the majority prostate cancer cell lines tested. Further analysis of resistant cell lines revealed that VCaP and TRAMP-C1 were still able to mount an IFN-I induced antiviral response, while defects in the IFN-I signalling pathway were found in VSV-GP susceptible cell lines. Results in cell lines were confirmed in primary cultures derived from patients who had undergone radical prostatectomy. In our in vivo studies, VSV-GP was able to cure Du145 subcutaneous tumours in a xenograft model and was able to slow down tumour growth in a TRAMP-C1 subcutaneous syngeneic model, significantly increasing life expectancy of VSV-GP treated mice. Since TRAMP-C1 are highly responsive to IFN-I signalling, we used two different approaches to improve therapy outcome, either a combination therapy with the Jak1/2 inhibitor Ruxolitinib, or a knock down of the IFNAR1 gene in TRAMP-C1 cells. However, neither of these approaches resulted in an improved outcome.
VSV-GP is a promising novel therapeutic for the treatment of prostate cancer. To optimize the efficiency of VSV-GP, further studies will be necessary to better understand how the oncolytic effect, the IFN-I response and anti-tumour immune response interact and what strategies will result in enhanced therapeutic outcome.

Poster # 1 cell biology

Unconventional farnesylation is essential for protein-protein interaction dependent kinetochore localization of human Spindly

Georg Altenbacher[1], Friederike Finsterbusch[1,4], Marin Barisic[1,3], Leopold Kremser[2], Herbert Lindner[2], Stephan Geley[1]

[1]Div. of Molecular Pathophysiology, Biocenter, Innsbruck Medical University, Innsbruck, Austria
[2]Div. of Clinical Biochemistry, Biocenter, Innsbruck Medical University, Innsbruck, Austria
[3]Cell Division Laboratory, Danish Cancer Society Research Center, Copenhagen, Denmark
[4]Institute of Physiological Chemistry, TU Dresden, Dresden, Germany

georg.altenbacher@i-med.ac.at

Spindly is part of the KNTC1/Rod-ZW10-ZWILCH (RZZ) complex that recruits cytoplasmic dynein to kinetochores during prometaphase. Spindly harbours a conserved C-terminal domain (CPQQ) that is required, but not sufficient, for kinetochore localization. Spindly was found to interact with protein farnesyltransferase and to be farnesylated at Cys602 in mitosis but not interphase. Interestingly, farnesylated Spindly was not processed by C-terminal cleavage and cysteine methylation. Consensus farnesylation sequences like CVLS could target Spindly to the kinetochore while a geranyl-geranylation motif (CAAL) led to mislocalisation. Depletion of the prenyl precursor mevalonate from cells prevented Spindly interaction with the RZZ complex and localisation to kinetochores. This defect could be rescued by addition of mevalonate or farnesyl pyrophosphate but not geranyl-geranyl pyrophosphate. Farnesylation was required for Spindly to bind to the RZZ complex, suggesting that non-canonical farnesylation is a novel protein-protein interaction means. Preventing nuclear localization of Spindly in interphase using a ΔNLS mutant produced a constitutively farnesylated but not processed Spindly indicating that Spindly farnesylation is cell cycle dependent, requires nuclear envelope breakdown and that CPQQ prevents cleavage via Rce1.

Alterations in epidermal eicosanoid metabolism contribute to inflammation and impaired differentiation in FLG mutated AD

cell biology Poster # 2

Loss-of-function mutations in filaggrin (FLG) cause ichthyosis vulgaris (IV) and represent the major predisposing genetic risk factor for atopic dermatitis (AD). While both conditions are characterized by epidermal barrier impairment, only AD exhibits signs of inflammation. This work aimed at delineating the role of FLG loss-of-function mutations on eicosanoid metabolism in IV and AD. Using epidermal equivalents (HEEs) generated with keratinocytes isolated from non-lesional skin of patients with FLG wild type AD (WT/WT), FLG mutated AD (FLG/WT), IV (FLG/FLG) or FLG WT control skin, we assessed the potential autocrine role of epidermal-derived eicosanoids in FLG- associated vs. FLG-WT AD pathogenesis. Ultrastructural analyses demonstrated abnormal SC lipid architecture in AD and IV HEEs, independent of FLG genotype. Both AD (FLG/WT) and IV HEEs showed impaired late epidermal differentiation, without impairment of the outside-in permeability barrier. Only AD (FLG/WT) HEEs exhibit significantly increased levels of inflammatory cytokines. Analyses of lipid mediators revealed increased arachidonic acid (AA) and 12-LOX metabolites levels. Treatment of control HEEs with AA increased expression of inflammatory cytokines, while 12-HETE attenuated expression of differentiation markers. Thus, FLG mutations lead to alterations in epidermal eicosanoid metabolism that could serve as an autocrine trigger of inflammation and impaired epidermal differentiation in AD.

Stefan Blunder[1], R.Rühl[2,3], V. Moosbrugger-Martinz[1], C. Krimmel[1], A. Geisler[1], D. Crumrine[4], P.M. Elias[4], R. Gruber[1,5], M. Schmuth[1], S. Dubrac[1]

[1]Department of Dermatology, Venereology and Allergology, Medical University of Innsbruck, Innsbruck, Austria
[2]MTA-DE Public Health Research Group of the Hungarian Academy of Sciences, Faculty of Public Health, University of Debrecen, Debrecen, Hungary
[3]Paprika Bioanalytics, Debrecen, Hungary
[4]Department of Dermatology, University of California, San Francisco, California
[5]Division of Human Genetics, Medical University of Innsbruck, Innsbruck, Austria

stefan.blunder@gmail.com

How are set the gating properties of L-type calcium channels?

Pierre Costé de Bagneaux[1], Marta Campiglio1, Bruno Benedetti[1], Petronel Tuluc[2], Bernhard E. Flucher[1]

[1]Dept. Physiology and Medical Physics, Medical University Innsbruck
[2]Department of Pharmacology and Toxicology, Medical University Innsbruck

pierre.coste@i-med.ac.at

Voltage-gated calcium channels regulate fundamental functions of muscle, nerve and endocrine cells. They are organized in four homologous repeats (I-IV) of six transmembrane domains (S1-S6). The four S5-S6 domains form the channel pore, whereas S1-S4 of each repeat forms separate voltage sensing domains (VSD). Among calcium channels, CaV1.1 is atypical because of its low voltage sensitivity and its slow activation kinetics. Our team identified an embryonic splicing variant (CaV1.1e) lacking exon 29 located between S3 and S4 of the repeat IV. This channel has increased voltage sensitivity and a higher current density, suggesting that exon29 modulates the mechanism controlling voltage sensitivity. Using structural modelling combined with mutagenesis and electrophysiology, our team recently discovered that the mechanism responsible for the increased voltage sensitivity of CaV1.1e involves interactions between aspartate D4 of IVS3 and two arginines R1 and R2 of IVS4. The presence of exon29 in the adult variant (CaV1.1a) disrupts this interaction, resulting in the poor voltage sensitivity. On the other hand, previous work from our and other laboratory indicated that the specific slow activation kinetics of CaV1.1a currents is determined within the I VSD. To understand how regulation of voltage-dependence of activation relates to regulation of activation kinetics, whether exon 29 by itself has the potential of curtailing CaV1.1 currents, and whether the proposed D4-R1/R2 interaction is specific to the IV VSD or can also function in the I VSD, we built multiple chimeric channels by swapping segments between the I VSD and IV VSD. Electrophysiological analysis of these chimeras demonstrated that exon 29 requires the specific interaction of IVS3 and IVS4 but has no modulatory activity on its own. IVS4 could not function in combination with IS3 but specifically requires IVS3. Moreover we showed that the integrity of the VSD I is essential for the slow activation kinetics of CaV1.1a and that its control could not be transferred to the IV VSD. Given that the residues involved in the D4-R1/R2 interaction are also present in other L-type calcium channels, we hypothesize that voltage sensitivity in the CaV family members is also driven by the same interaction. To investigate whether the corresponding residues control the voltage sensitivity in the cardiac channel, we constructed the corresponding point mutations in CaV1.2 and are currently analysing their gating properties. Our preliminary results suggest that D4 the IV VSD also controls the voltage sensitivity of the cardiac channel.

Development of opto-FGFR1 constructs with different subcellular targeting domains

Katalin Csanaky, Lars Klimaschewski

Division of Neuroanatomy, Department of Anatomy and Histology, Medical University of Innsbruck, Austria

katalin.csanaky@i-med.ac.at

The murine opto-fibroblast growth factor receptor 1 (opto-FGFR1) is a chimeric receptor consisting of the catalytic domain of mammalian receptor tyrosine kinase and the algal light-oxygen-voltage-sensing (LOV) domain. It is anchored to the plasma membrane with an N-terminal myristoylation or a p75 transmembrane sequence. The light-induced LOV domain dimerization results in transphosphorylation of the receptor kinase domains followed by activation of the main signaling pathways, such as MAPK/ERK, AKT and PLCγ providing the possibility to control cellular FGFR activity with spatial and temporal precision.

Our aim was to design opto-FGFR constructs, which are localized to the endosomes and nucleus, to investigate the activation of FGFR1 in different subcellular compartments. Human embryonic kidney 293 (HEK293), U373 glioblastoma and PC12 pheochromocytoma cells were transfected with Lipofectamin 2000 or Amaxa nucleofection. For light stimulation an ExoTerra incubator was equipped with 300 light-emitting diodes. The blue light intensity was measured with a digital powermeter and the intensity was set to 2.5μW/mm2. To investigate the FGFR signaling pathway activation FRET based biosensor of ERK activity (EKARcyto), Elk-1-luciferase assay, immunocytochemistry and Western blots were applied. Our results showed that we successfully engineered nuclear and endosomal opto-FGFR, containing three nuclear localization signals (NLS) or two PI(3)P-binding FYVE finger domain sequences. By confocal laser scanning we proved that the designed mVenus tagged opto-FGFRs are localized at the desired subcellular compartments in transfected HEK293 cells. After 8-hours blue light stimulation elevated MAPK activity was measured by luciferase assay in HEK293 cells transfected with any of the three opto-FGFRs. Western blots following 5-min blue light stimulation increased the phosphorylation of pERK and pAKT. Enhanced FRET signal was observed after NGF treatment in cytosolic EKAR transfected PC12 cells.

From our preliminary data we conclude that three blue light sensitive, fluorescently-tagged and functionally active opto-FGFRs are available, localized in the plasma membrane, endosomes and in the nucleus. Our further aim is to compare the effect of the membrane, endosomal and nuclear localized FGFRs on PC12 cells differentiation and on axon outgrowth by primary neurons in vitro.

Functional Analysis of Rbm26

Ana Curinha[1], Katharina Klee[1], Giridhar Shivalingaiah[1], Pia Anstaad[2], Stephan Geley[1]

[1]Division of Molecular Pathophysiology, Biocenter, Medical University Innsbruck,
Innsbruck, Austria
[2]Institute of Molecular Biology, University of Innsbruck, Innsbruck, Austria

ana.curinha@i-med.ac.at

Background: RNA binding motif protein 26 (Rbm26) is a conserved but poorly characterized protein that contains a PWI and two RBM domains as well as a Zinc finger domain. The Drosophila Rbm26 homolog, Swm, is an essential gene involved in cell cycle control and Hedgehog signal transduction.
Preliminary analysis of Rbm26 function in Zebrafish and human cells suggested roles in cell cycle regulation, ciliogenesis and development.
Questions and aims:
1. What is the subcellular localization of Rbm26/27 and do these proteins undergo cell cycle dependent changes in localization or abundance?
2. Are Rbm26/27 essential for cellular proliferation or ciliogenesis in human cells?
3. With which proteins and RNAs does Rbm26/27 interact?
To better understand the function of human Rbm26 and Rbm27 we expressed them as GFP fusion proteins and could detect them in small nuclear speckles confirming the localization of endogenous Rbm26 as determined by immunofluorescence studies. Rbm26 protein levels were found to oscillate, showing an expression peak during early S-phase. Knockdown of Rbm26 by RNAi impaired proliferation of human Hela and RPE cells. Crispr-Cas9 mediated deletion of Rbm26 in human Hela cells revealed that Rbm26 also caused a mild proliferation defect. Rbm26 was also found to be essential for primary cilia formation in serum starved RPE cells.
These results suggest that human Rbm26 might be an important regulator of ciliogenesis, similar to results obtained in Zebrafish. Whether Rbm26 directly affects ciliogenesis or acts via modulation of cell cycle control is currently under investigation. In addition, we have established a Rbm26 expressing cell line suitable for tandem affinity purification of Rbm26 to define interacting proteins and mRNA molecules.

Retinoic Acid in Myocardial Infarction. The Good? The Bad? The Ugly!

Katarina Danzl, Katharina Heinz, Christian Doppler, David Bernhard

katarina.danzl@i-med.ac.at

Objective: 5-Methoxyleoligin (5ML), a lignan from Edelweiss improves heart function of infarcted rat hearts by 21%. Preliminary studies suggested an involvement of all-trans retinoic acid signalling (ATRA) in this 5ML mediated effect. Importantly, recent in vivo studies revealed that ATRA physiologically increases in response to MI. Still, the role of ATRA in myocardial infarction remained totally unclear to date. In this study, we aimed to reveal the cardio-protective mechanisms of 5ML and give new insights in ATRA - induced pathophysiological mechanisms after MI.
Material and Methods: RARE luciferase reporter gene assays in Human Umbilical Vein Endothelial Cells (HUVECs) were performed. Cell death upon hypoxia was assessed using FACS analysis in 5ML, ATRA or combinational-treated cells. Western Blot analysis revealed the involvement of proteins important in oxidative stress response. Therefore, reactive oxygen species (ROS) production was determined using FACS based technologies.
Results: We show that ATRA induced luciferase signalling can be completely abolished by 5ML treatment. This ATRA reducing potential of 5ML was also confirmed by HPLC and western blot analyses. Importantly, we exclusively show that ATRA promotes hypoxia induced cell death of HUVECs, which is reversed by co-treatment with 5ML. TXNIP, a negative regulator of the oxidative stress neutralizing enzyme thioredoxin has been found to be upregulated by retinoic acid signalling. In line with these findings reactive oxygen species production is increased upon ATRA treatment. Co-treatment with 5ML not only reverses all these negative effects but also leads to a sustained cell survival. Notably, we identified ATRA as a key-molecule in 5ML mediated effects, because these effects can be partially ameliorated by overexpression of the ATRA induced stress mediator TXNIP.
Discussion: With this study we reveal new insights in the pathophysiological role of ATRA in hypoxia and myocardial infarction. In other studies systemically applied ATRA has been found to have protective effects on cardiovascular disease. Here, we show that locally increased retinoic acid signalling leads to an obstructed oxidative stress response via overexpression of TXNIP and cell death in hypoxia. 5ML reduces ATRA level, therefore prevents cells from O2 stresses and leads to sustained cell survival.

Physiological role of α2δ subunits in GABAergic synapses of cultured medium spiny neurons

Stefanie Geisler, Clemens L. Schöpf, Ruslan Stanika, Larissa Traxler, Gerald J. Obermair

stefanie.geisler@i-med.ac.at

Auxiliary α2δ subunits modulate membrane trafficking and current properties of voltage-gated calcium channels (VGCCs) and have recently been implicated in synaptic transmission and synapse formation. Three of the four isoforms (α2δ-1, α2δ-2, and α2δ-3) are stably expressed in the CNS; however, how the individual isoforms contribute to specific functions in neurons simultaneously expressing all isoforms is largely elusive. By establishing a cellular α2δ triple knock-out model in cultured hippocampal neurons, we could recently identify α2δ subunits as key regulators of glutamatergic synaptogenesis (see poster by Schöpf et al.). In contrast, synapses of the occasionally observed GABAergic neurons were unaffected by knockout of all three subunits and thus the role of α2δ subunits in GABAergic synapse formation remains unclear. A puzzling observation, however, indicates specific functions of presynaptic α2δ subunits in inhibitory synapses: postsynaptic GABAA-receptors were specifically upregulated opposite glutamatergic boutons in neurons overexpressing α2δ-2.

Therefore in order to study α2δ subunits in GABAergic synapses we established cultured striatal neurons, which consist of about 95-98% inhibitory medium spiny neurons (MSN). Most importantly, quantitative RT-PCR analysis revealed that the striatum expresses all three neuronal α2δ subunits with α2δ-3 being the dominant isoform. Addressing the functions of α2δ subunits required the prior characterization of synapses formed by and innervating cultured MSNs using immunofluorescence and patch clamp electrophysiology.

In line with previous observations the proper maturation of dendritic arbors and spines of MSNs required the co-culture with glutamatergic cortical neurons. Co-cultured MSNs started to express Darpp-32 (dopamine- and cAMP-regulated phosphoprotein of 32 kDa) at around DIV7 and the percentage of Darpp-32 positive neurons continuously increased until DIV21. This molecular specification was paralleled by the development of complex dendritic arbors and spines. Lentiviral infection of MSNs with soluble eGFP just before starting the co-culture allowed discriminating MSN from cortical neurons.

As expected, immunofluorescence analysis of the vesicular GABA transporter (vGAT) revealed GABAergic synapses formed on dendritic shafts of neighboring neurons. Furthermore, a specific labeling of PSD-95 in spine heads opposite to presynaptic terminals formed by cortical neurons, demonstrated the presence of excitatory synapses on MSN spines. The functionality of GABAergic and glutamatergic synapses was confirmed by current clamp recordings of mEPSCs and mIPSCs in 14DIV old neurons.

In order to study whether calcium channel α2δ subunits affect the composition of GABAergic synapses, we labeled MSNs transfected with individual α2δ isoforms together with eGFP for pre- and postsynaptic components (vGAT/GABAAR β2/3 subunit). Qualitative analysis indicated an increased density of postsynaptic GABAA-receptors opposite presynaptic boutons of α2δ-2 overexpressing MSNs when compared with control (eGFP) or α2δ-1 expressing neurons. Therefore our preliminary findings suggest that presynaptic α2δ subunits determine the postsynaptic composition and thus strength of GABAergic synapses in an isoform specific manner.

Investigation of the molecular mechanisms underlying cell death upon extended mitotic arrest

Cell death on extended mitotic arrest is considered arguably most critical for the efficacy of microtubule-targeting agents (MTAs) in anticancer therapy. While the molecular machinery controlling mitotic arrest on MTA treatment, the spindle assembly checkpoint (SAC), appears well defined, the molecular components executing cell death, as well as factors connecting both networks remain poorly understood. We conducted a mini screen exploring systematically the contribution of individual BCL2 family proteins at single cell resolution to death on extended mitotic arrest, and demonstrated that the mitotic phosphorylation of BCL2 and BCLX represent a priming event for apoptosis that is ultimately triggered by NOXA-dependent MCL1 degradation, enabling BIM-dependent cell death. Our findings provide a comprehensive model for the initiation of apoptosis in cells stalled in mitosis and provide a molecular basis for the increased efficacy of combinatorial treatment of cancer cells using MTAs and BH3 mimetics. For the future we plan to focus our work on the regulation of NOXA to answer the question what defines NOXA stability and – in extension – MCL1 turnover.

Manuel Haschka[1], Claudia Soratroi[1], Susanne Kirschnek[2], Georg Häcker[2], Richard Hilbe[3], Stephan Geley[3], Andreas Villunger[1], Luca Fava[1]

[1]Division of Developmental Immunology, Medical University Innsbruck, Innsbruck, Austria, [2]Institute for Medical Microbiology and Hygiene, Freiburg, Germany [3]Division of Molecular Pathophysiology, Innsbruck, Austria

manuel.haschka@i-med.ac.at

Poster # 9 cell biology

Screening of a plant-derived small molecule database for potential CYP2D6 inhibition, using in-silico based pre-selection and depiction as well as in-vitro refinement

Johannes Hochleitner[1], M. Naschberger[1], D Schuster[2], F Überall[1]

[1]Division of Medical Biochemistry Center for Chemistry and Biomedicine, Medical University Innsbruck, Austria
[2]Division of pharmaceutical chemistry, computer aided molecular design group, University of Innsbruck, Austria

johannes.hochleitner@i-med.ac.at

The Cytochrome P450 enzyme (CYP) complex is encoded by 57 functional genes that are divided into 18 families and 44 subfamilies according to their sequential similarity. From all phase I drug metabolizing enzymes (DMEs) that are able to functionalize xenobiotics; CYPs comprise for up to 80 % and are therefore important targets for drug-drug interactions.
Isoenzyme cytochrome P450 2D6 (CYP2D6) is regarded as highly polymorphic, which results in varying amounts of functional protein levels in the detoxifying organs of the human body. In contrast to other CYPs, its expression is not affected by sex, age or drugs and metabolizes up to 25% of all prescribed drugs, especially analgesics, beta blockers, the anticancer drug Tamoxifen, as well as anti-arrhythmics, -psychotics and -depressants.
As co-medication with phytotherapeutics is a therapeutic trend among patients it is of utmost importance to know the interaction potential of phytochemicals with the detoxifying organs of the human body.
To get a better understanding of the inhibition types of phytochemicals on CYP2D6, we use a combinatorial approach of in-silico and in-vitro methods in order to exploit the strengths and eradicate the weaknesses of the particular test method.
Our main goal is to create a robust workflow for generating CYP2D6 inhibition data for phytochemicals. In order to assure a low false-positive error rate, we scanned a database that consists of plant derived small molecules with a pharamcophore model. This previously published in-silico tool helped us to identify new inhibitors, as it filters compounds from the database that share the same molecular properties (i.e. plane-, ionisability-, hydrophobicity- and acceptor-features) like established inhibitors. After passing this first check point, the prospective inhibitors were applied on a luminescence based high-throughput CYP2D6 inhibition assay in-vitro, in order to generate dose-response curves. As it is known that high-throughput assays show weaknesses (i.e. unspecific enzyme binding and fluorescence quenching) if challenged with phytochemicals, we consider this a crucial step serving as second checkpoint. With profound knowledge of the molecular constitution and behaviour in high-throughput CYP2D6 inhibition assays, we plan to bring the compounds in this third step back in-silico, in order to predict their binding poses in the CYP2D6 active site cavity. For this last checkpoint we created a docking model based on the protein crystal structure of CYP2D6, co-crystallized with an inhibitor.
Thus, we present a workflow with three checkpoints which combines two in-silico models that enrich, rather than hinder each other and an in-vitro validation method that outlines the complexity of collecting reliable CYP2D6 inhibition data.

Nucleotide modifications within bacterial messenger RNAs regulate their translation and are able to rewire the genetic code

cell biology Poster # 10

Nucleotide modifications within RNA transcripts are found in every organism in all three domains of life. 6-methyladeonsine (m6A), 5-methylcytosine (m5C) and pseudouridine (Ψ) are highly abundant nucleotide modifications in coding sequences of eukaryal mRNAs, while m5C and m6A modifications have also been discovered in archaeal and bacterial mRNAs. Employing in vitro translation assays, we systematically investigated the influence of nucleotide modifications on translation. We introduced m5C, m6A, Ψ or 2´-O-methylated nucleotides at each of the three positions within a codon of the bacterial ErmCL mRNA and analyzed their influence on translation. Depending on the respective nucleotide modification, as well as its position within a codon, protein synthesis remained either unaffected or was prematurely terminated at the modification site, resulting in reduced amounts of the full-length peptide. In the latter case, toeprint analysis of ribosomal complexes was consistent with stalling of translation at the modified codon. When multiple nucleotide modifications were introduced within one codon, an additive inhibitory effect on translation was observed. We also identified the m5C modification to alter the amino acid identity of the corresponding codon, when positioned at the second codon position. Our results suggest a novel mode of gene regulation by nucleotide modifications in bacterial mRNAs.

Thomas P. Hoernes[1], Nina Clementi[1], Klaus Faserl[2], Heidelinde Glasner[3], Kathrin Breuker[3], Herbert Lindner[2], Alexander Hüttenhofer[1], Matthias D. Erlacher[1]

[1]Division of Genomics and RNomics, Biocenter, Medical University of Innsbruck, Innsbruck, Austria
[2]Division of Clinical Biochemistry, Biocenter, Medical University of Innsbruck, Innsbruck, Austria
[3]Institute of Organic Chemistry and Center for Molecular Biosciences (CMBI), University of Innsbruck, Innsbruck, Austria

thomas.hoernes@i-med.ac.at

Study the function and biological significance of HAT1 in *Drosophila melanogaster*

Anming Huang[1], Lukas Trixl[1], Herbert Lindner[2], Leopold Kremser[2], Alexandra Lusser[1]

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

anming.huang@i-med.ac.at

CENP-A is a histone H3 variant that is deposited at the centromeric region of a chromosome. The special chromatin architecture of the centromere enables the assembly of the kinetochore, which is required for microtubule attachment and faithful segregation of sister chromatids during cell divisions. CENP-A proteins are rather divergent from other H3 variants and have limited similarity to each other from different species. CENP-A is incorporated into chromatin in a replication-independent fashion, while the question of precisely how CENP-A is targeted to centromeres and loaded in a cell cycle-specific manner is not completely understood. Multiple studies in different organisms have revealed that a complex network of factors is required to ensure timely and spatially constrained incorporation. However, there are still large gaps to our understanding of CENP-A/Cid loading pathways in Drosophila.
Our group found there are at least three different CENP-A preloading complexes in Drosophila. Two complexes contain the CENP-A chaperones CAL1, FACT and/or Caf1/Rbap48. One novel complex consists of the histone acetyltransferase Hat1, Caf1 and CENP-A/H4. CENP-A/Cid interacts with the HAT1 complex via an N-terminal region and which is acetylated in cytoplasm but not in nuclei, these suggest a histone acetyltransferase activity-independent escort function for Hat1.
I analysed the subcellular distribution of CENP-A and Hat1 proteins and showed that Hat1 is required for proper CENP-A loading into chromatin. Based on these findings, my future research work is to address the following questions: First, do different posttranscriptional modifications mark CENP-A in different preloading complexes and what are their roles? Second, which region of HAT1 is needed to interact with CENP-A and what is the manner of interaction in the HAT1-CENP-A complex? Third, what is biological role of HAT1 in *Drosophila melanogaster*?

Atypical transcriptional switching via TCF/β-catenin for germ layer segregation

Willi Kari, Lena Zitzelsberger, Patrick Lemaire, Yutaka Satou, Vincent Bertrand, Ute Rothbächer

willi.kari@uibk.ac.at

In ascidians, it is well established that vegetal accumulation of ß-catenin suppresses the animal fate. In Ciona, we previously showed that β-catenin/TCF dependent repression of GATAa transcription factor activity mediates germ layer segregation (Rothbächer et al, 2007). Moreover, tight temporal correlation of ß-catenin accumulation with GATA activity repression is consistent with direct repression of GATAa target genes. A GATA site multimer is similarly repressed, indicating that GATA factor binding sites are responsive to this repression and that canonical TCF binding sites (normally mediating activation) are not involved. Indeed, atypical TCF sites were linked to ß-catenin/TCF mediated repression in Drosophila (Blauwkamp et al., 2008). In addition, we very recently described a direct repressive TCF/ß-catenin mechanism in *C. elegans* on other transcription factor binding sites (Murgan, Kari et al., Dev. Cell, in press). In ascidian germ layer segregation, GATAa target genes may be directly repressed by ß-catenin/TCF and we are currently testing the hypothesis that atypical repressive ß-catenin/TCF signaling works through GATA sites in germ layer formation.
To investigate the repressive activity of ß-catenin/TCF at GATA sites, we separated atypical TCF sites from GATA sites by generating activity reporter constructs (G12 constructs) with different GATA binding site composition. We found that their GATA responsiveness differed in the animal region which however did not always correlate with variably increasing vegetal repression presumably by maternal β-catenin/TCF suggesting a “repressive GATA signature” in germ layer segregation.
Presently, we are analyzing the biochemical interaction of GATAa/TCF/ß-catenin on “repressive” GATA sites for atypical transcriptional switching in Ciona.

A novel approach for the prevention ROS-induced ischemia re-perfusion injuries (IRI) in solid organ transplantation

Sana Khalid1, A. Drasche[1], M. Thurner[1], M. Hermann[2], M. Imtiaz Ashraf[1], F. Fresser[3], G. Baier[3], L. Kremser[4], H. Lindner[4], J. Troppmair[1]

[1]Daniel Swarovski Research Laboratory, Department of Visceral, Transplant and Thoracic Surgery
[2]Department of Anesthesiology and Critical Care Medicine
[3]Department for Pharmacology and Genetics, Division of Translational Cell Genetics
[4]Division of Clinical Biochemistry, Protein Micro-Analysis Facility, Biocenter, Medical University of Innsbruck, Innsbruck, Austria

sana.khalid@i-med.ac.at

p66Shc produced reactive oxygen species (ROS) contribute to many pathologies including IRI during solid organ transplantation. A therapeutic approach is to target the signaling cascades involved in ROS production as antioxidants yielded little clinical benefits. Previous work suggested that pro-oxidant and pro-apoptotic function of p66Shc required mitochondrial import which depended on serine 36 (S36) phosphorylation. PKCβ has been identified initially as S36 kinase but c-Jun N-terminal kinases (JNKs) may also phosphorylate this residue, with unclear consequences for ROS production and cell death. We could confirm PKCβ mediated ROS regulation but not p66ShcS36 phosphorylation.
We showed under prooxidant treatment and hypoxia reoxygenation (HR) in mouse embryonic fibroblast (MEF) and HL-1 cardiomyocytes that inhibition of MAPKs decreased p66Shc S36 phosphorylation, ROS production and cellular damage only for JNK, which also directly interacted with p66Shc. We further confirmed these findings in JNK1/2 deficient MEFs. Finally, the low ROS phenotype of JNK1/2 knockout MEFs was reversed by the phospho-mimetic p66ShcS36E mutant. In search for the PKCβ phosphorylation sites in p66Shc mass spectrometric (MS) analyses revealed S139 as PKCβ regulatory site. p66Shc-deficient MEFs reconstituted with the S139A mutant of p66Shc showed significantly reduced mitochondrial ROS levels and cell death. We thus propose a model, there by targeting upstream activators (PKCβ, JNK) of p66Shc may provide a therapeutic approach for decreasing mitochondrial-ROS mediated organ damage.

LAMTOR2 in regulation of fat metabolism

Gudrun Liebscher[1], Giorgia Lamberti[1], Cedric H. De Smet[1], Caroline Herrmann[1], Nemanja Vujic[2], Dagmar Kratky[2], Lukas A. Huber[1]

[1]Biozentrum der Medizinische Universität Innsbruck, Innsbruck, Austria
[2]Institute of Molecular Biology and Biochemistry, Medical University of Graz, Graz, Austria

gudrun.liebscher@i-med.ac.at

LAMTOR2 is part of the LAMTOR complex anchored to late endosomal/lysosomal membranes. The LAMTOR complex is known to regulate mTORC1 signaling in an amino acid dependent manner and MAPK signaling. Both signaling pathways play a crucial role in cellular homeostasis. Deletions of LAMTOR components are embryo-lethal in mice, but conditional knock outs allow the study of the complex. Microarray and proteomic studies in LAMTOR2 -/- mouse embryonic fibroblasts (MEF) and macrophages point to a regulation of lipid metabolism including lipid synthesis, uptake, transport and degradation. Furthermore the LAMTOR complex seems to play an important role in adipocyte differentiation since LAMTOR2 -/- MEFs are deficient in adipogenesis (our own unpublished results, de Smet et al.).
To study the regulation of lipid metabolism in more detail an adipose tissue specific AdipoqLAMTOR2 -/- mouse line was generated. These mice show an accumulation of lipids e.g. triglycerides in the blood, brown adipose tissue (BAT) and liver. This could be a result of the lower mitochondrial activity in the BAT in which the amount of mitochondria is unchanged.
Additionally, starvation and refeeding of AdipoqLAMTOR2-/- mice shows a defect in activating mTORC1 signaling and a deregulation of the lipid homeostasis regulator SREBP1.
Although the marker of thermogenesis Ucp1 is downregulated at room temperature in AdipoqLAMTOR2-/- mice, acute cold treatment leads to reduced triglyceride levels in the blood and BAT in both the AdipoqLAMTOR2 -/- and AdipoqLAMTOR2 flox/flox mice. Interestingly, this reverses the phenotypes observed in BAT and blood.
In summary, an adipose tissue specific knock out of LAMTOR2 disrupts brown adipose tissue homeostasis and has effects on the whole body lipid metabolism.

Scaffolding functions of GPCRs

Johanna Mayrhofer[1], V.A. Bachmann[1], R. Ilouz[2], R. Röck[1], PM Tschaikner[3], P. Raffeiner[1], P. Aanstad[3], S.S. Taylor[2], E. Stefan[1]

[1]Institute of Biochemistry and Center for Molecular Biosciences, University of Innsbruck, Austria
[2]Department of Pharmacology and Department of Chemistry and Biochemistry, University of California, San Diego, United States
[3]Institute of Molecular Biology, University of Innsbruck, Austria

johanna.mayrhofer@uibk.ac.at

Signal transmission emanating from the G protein-coupled receptor (GPCR) family to intracellular effector cascades is organized by pleiotropic scaffolding proteins. Signaling scaffolds such as A-kinase anchoring proteins (AKAPs) compartmentalize kinase activities and ensure substrate specificity [1]. In previous studies we have identified dynamic protein:protein interactions (PPI) of compartmentalized protein kinase A (PKA) [2] with distinct molecular switches downstream of receptor cascades [3,4]. In order to gain a more comprehensive and mechanistic understanding of cAMP-controlled macromolecular PKA complexes we chose a phospho-proteomics approach to map dynamic PPIs. We affinity-purified endogenous PKA complexes from osteosarcoma cells and generated a dynamic PPI network. Besides well-known connections to AKAPs, we identified possible links of PKA to metabolic pathways, protein transport, RNA binding, GTP binding, calcium signaling, and nuclear signaling. We selected and investigated a novel PPI between the cAMP/PKA cascade and an orphan GPCR, the GPR161, which has been shown to be involved in Hedgehog signaling [5]. We show that the orphan GPCR GPR161 contains the structural features to function as selective high-affinity type I AKAP. Binary complex formation of GPR161 exclusively with type I PKA regulatory subunits affect plasma membrane targeting in cells and provokes GPR161-mediated PKA recruitment into the primary cilium of zebra-fish embryos. We illustrate that receptor-anchored PKA complexes enhance cAMP-mediated GPR161 phosphorylation, which is regarded as one general principle for regulating GPCR desensitization. In addition we revealed that distinct 'rare disease' mutations of PKA regulatory subunits differentially contribute to spatially restricted interactions of GPR161-anchored PKA type I holoenzyme complexes. We propose that the ciliary GPR161-PKA signalosome is a compartmentalized signaling hub that directly integrates receptor-sensed input signals with spatiotemporal cAMP dynamics.

The Monothiol Glutaredoxin GrxD is Essential for Growth and Iron Homeostasis in *Aspergillus fumigatus*

Matthias Misslinger[1], K. Mair[1], N. Beckmann[1], E.R. Werner[2], H. Haas[1]

[1]Department of Molecular Biology, Medical University of Innsbruck, Austria
[2]Division of Biological Chemistry, Biocenter, Innsbruck Medical University, Austria

matthias.misslinger@i-med.ac.at

Efficient adaptation to iron starvation is an essential virulence determinant of the most common airborne fungal pathogen *Aspergillus fumigatus*. In the current study we characterized the role of the *A. fumigatus* monothiol glutaredoxin ortholog GrxD (Afu2g14960), orthologs of which mediate cellular transport and sensing of iron in *S. cerevisiae* and *S. pombe*.
Heterokaryon rescue technology proved that grxD is an essential gene in *A. fumigatus*, which contrasts *S. cerevisiae* and *S. pombe*. Conditional expression of grxD demonstrated that GrxD deficiency can be partially compensated by high iron supplementation. Taken together with the transcriptional upregulation of grxD during iron starvation, these data are in agreement with a role of GrxD in cellular iron transport. In a shift from GrxD-inducing to -repressing conditions under iron starvation, the grxDc strain displayed increased accumulation of protoporphyrine IX (PpIX), the iron-free precursor of heme. This is consistent with a role of GrxD in iron sensing as PpIX accumulation is a hallmark of deficiency in HapX, the major transcription factor required for adaptation to iron starvation. However, deregulation of iron-regulated genes at the transcriptional level was not observed. Moreover, we identified a strain, which carries a grxDc suppressor mutation enabling growth in standard medium. Transcriptional analysis combined with genetic mapping revealed that the suppressor phenotype is caused by derepression of iron uptake via inactivation of sreA, which encodes a transcriptional repressor of siderophore biosynthesis and reductive iron assimilation.

Functional properties of newly identified somatic CACNA1D mutations in aldosterone-producing adenomas

Giulia Negro[1], A. Pinggera[1], P. Tuluc[1], S. Monteleone[2], K. Liedl[2], J. Striessnig[1]

[1]Institute of Pharmacy and Center for Molecular Biosciences; University of Innsbruck; Innsbruck, Austria
[2]Institute of General, Inorganic and Theoretical Chemistry and Center for Molecular Biosciences; University of Innsbruck; Innsbruck, Austria

giulia.negro@uibk.ac.at

Background: Almost 5 % of hypertension is caused by aldosterone-producing adenomas (APAs). We have previously identified seven new mutations occurring in the pore-forming α1 - subunit (CACNA1D gene) of Cav1.3 voltage-gated Ca2+ channels. For four of them (G403R, I750M, P1336R and V259D) we identified a gain-of-function phenotype which explains excess of aldosterone production. Here we report the functional characterization of the three APA mutations not studied so far (F747L, R990H and M1354I) to determine if they also enhance calcium entry through these channels.
Methods: TsA-201 cells were transiently transfected with the cDNA of mutations F747L, M1354I and R990H in Cav1.3 α1 – subunits (long splice variant, Cav1.3L), together with the auxiliary β3 and α2δ subunits. Biophysical properties of Ca2+ inward currents (15 mM Ca2+; ICa), were measured in whole-cell patch-clamp recordings. Western blot analysis was used to quantitate α1 protein expression levels.
Results: Mutation F747L severely affected Cav1.3L gating by significantly shifting V05,act (-13.9 mV, n > 15) and V05,inact (-4 mV, n > 7) to more negative voltages and by significantly slowing inactivation (remaining current [%] after 250 ms: 43.3 ± 2.25 %, n = 28 for WT, 70.6 ± 3.96 %, n = 14 for F747L, p-value < 0.001, unpaired Student's t-test). Moreover, ON-gating charge was not detectable in mutant channels suggesting also enhanced open probability. In contrast, mutation M1354I did not show significant differences in channel gating (V05,act, V05,inact), or inactivation time course. For the R990H mutant an increased ITail/QON ratio was observed, suggesting an enhanced open probability indicative of a gain-of-function phenotype. In addition, molecular modelling studies strongly suggest that this mutation affects a critical arginine residue in the S4 helix of the voltage sensor in domain III, thus creating an additional pore in this voltage sensor domain (VSD) permitting a depolarizing flow of protons ("ω-current") in the resting state. Total protein expression levels were increased for F747L and R990H mutations compared to wild-type Cav1.3L, but were unchanged for M1354I.
Conclusions: Mutation F747L results in strong gating changes compatible with a channel gain-of-function and therefore supports its disease-causing role in APAs. Molecular modelling of mutation R990H strongly points to a disease-relevant creation of a pore in the VSD, where ω-currents may induce depolarization of the cell. In contrast, the disease-causing potential role of M1354I could not be confirmed in our studies.

The Ca^{2+} -activated Cl-channel in zebrafish skeletal muscle is activated during excitation-contraction coupling

Shu Fun Josephine Ng, Anamika Dayal, Manfred Grabner

Division of Biochemical Pharmacology, Medical University Innsbruck, Austria

shu-fun-josephine.ng@i-med.ac.at

Ca2+-activated Cl- channels (CaCC) also known as TMEM16A or Anoctamin (Schroeder et al., Cell, 2008; Yang et al., Nature, 2008; Caputo et al., Science, 2008), are expressed in various tissues and play important roles in numerous physiological functions such as epithelial secretion, olfactory and sensory transduction, cardiac excitability, and smooth muscle contraction. Even though CaCC mRNA was identified in human skeletal muscle (Huang et al., 2006), no CaCC conductance has been reported till date. Surprisingly, we found robust CaCC currents with current amplitudes of >100 pA/pF at +80 mV membrane potential in zebrafish skeletal muscles. Utilising the zebrafish model, we aim to investigate whether CaCC is important for skeletal muscle EC coupling and if yes, the mechanism that it influences the skeletal muscle EC coupling.
We identified Ano1 (TMEM16A) as the major Anoctamin candidate responsible for this massive Cl- influx into zebrafish skeletal muscles by immunocytochemical stainings and subtype-sepcific CaCC current blockers. The mechanism of action of CaCC current blockers were investigated by whole-cell patch-clamp recordings of zebrafish myotubes. Biophysical characterisation of the CaCC current in skeletal muscle shows that the current is outwardly rectifying at sub-maximal Ca2+ levels and exhibits a linear current-voltage relationship at high [Ca2+], indicating that Ano1 in skeletal muscle is a bona fide CaCC. Interestingly, this robust CaCC current can only be observed in wild-type zebrafish myotubes, which display intact SR Ca2+ release during excitation-contraction (EC) coupling. In contrast, the CaV1.1 β1-null zebrafish mutant relaxed, lacking the SR Ca2+ release, displayed no CaCC current. Thus, the CaCC current through Ano1 is activated by SR Ca2+ release during EC coupling. Previous publication from our lab (Schredelseker et al., PNAS, 2010) demonstrated that unlike the mammalian system, zebrafish possesses non-conducting DHPR-α1S in skeletal muscles. To find out whether this DHPR non-conductivity is a prerequisite for proper CaCC functioning, whole-cell patch-clamp recordings were carried out on mammalian DHPR-α1S –expressing zebrafish myotubes. Surprisingly, in the presence of DHPR Ca2+ influx, we observed a reduction in SR Ca2+ release, which leads to a CaCC current reduction, a phenomenon which is different from what was reported in mammals.
Integrating the above findings, we hypothesized that the CaCC current in zebrafish skeletal muscle plays a role in shaping the action potential by shortening the repolarization phase and thus allowing faster muscle contraction. To test this hypothesis, we performed action potential recordings by current clamp electrophysiology on normal and relaxed myotubes. Interestingly, in relaxed myotubes in which CaCC current is absent, we observed a 44% broadening of action potential width at 1/3 spike height. This demonstrates that CaCC is vital for regulating action potential waveform, thus maintaining short action potential duration to ensure fast muscle contraction. Electrophysiological studies on our double transgenic zebrafish model (Dayal et al., in preparation) revealed a significant difference in the CaCC current size between the deep fast and superficial slow muscles. Further current-clamp studies, to see if this difference has an effect on the action potential properties in fast and slow muscles, are on the way.

Sprouty2, a modulator of receptor tyrosine kinase signaling in human glioma cells

Jongwhi Park[1], S. Geley[2], L. Klimaschewski[1]

[1]Division of Neuroanatomy, Innsbruck Medical University, Innsbruck, Austria
[2]Division of Molecular Pathophysiology, Innsbruck Medical University, Innsbruck, Austria

jongwhi.park@i-med.ac.at

Malignant glioma is among the most malignant human cancers. Despite the improved understanding of pathobiological features and novel therapeutic approaches, median survival of malignant glioma patients remains at 12 months. Dysregulation of receptor tyrosine kinase (RTK) signaling has been documented in a variety of cancers. Aberrant activation of the main signaling pathways such as RAS-ERK downstream from RTKs is frequently reported. Sprouty2 (Spry2) protein acts as a key regulator of RAS-ERK signaling. In breast, lung and liver cancer, Spry2 protein levels were significantly down-regulated compared to normal tissues and Spry2 re-expression attenuated the proliferative potential. On the other hand, in colon cancer Spry2 expression enhanced cellular proliferation and promoted metastasis. Accordingly, Spry2 mediated regulation occurs in a cell and context dependent manner.
In brain tumors, the function of Spry2 is unclear. In this project we aim 1) to elucidate the possible role of Spry2 in Glioblastoma multiforme (GBM) using human GBM cell lines and 2) to find out the detailed signaling pathways involved in Spry2 regulated RTK stimulation by modulating Spry2 expression. The project is based on molecular approaches such as viral expression systems, shRNA-mediated depletion of Spry2 and CRISPR/CAS induced Spry2 knock-out cells.
Our data show that Spry2 and eGFP cotransfected cells exhibit less BrdU incorporation compared to eGFP alone transfected cells. Consistent with the first result, Spry2 transduced cells by recombinant lentivirus grow slower than control transduced cells. Furthermore, this inhibitory effect is abolished in Spry2 dominant-negative mutant harbouring a substitution of tyrosine 55 with phenylalanine. By modulating Spry2 expression using titer-dependent transduction of adenovirus, we will evaluate again if ectopic Spry2 has an inhibitory effect on cell proliferation. In addition, Spry2 targeting short hairpin construction and CRISPR/CAS system allow us to down-regulate Spry2 levels. Together with overexpression studies, loss-of-function experiments will shed a light on the influence of Spry2 on the malignant phenotype of GBM cell lines. The results of this project may provide a molecular basis for novel therapeutic strategies targeting Spry2.

GRX2 as a useful tool to measure iron-sulfur-cluster formation in Friedreich's Ataxia

The autosomal recessive disease Friedreich's Ataxia (FA) is the most common inherited ataxia. Caused by an intronic GAA-trinucleotide expansion in the frataxin gene, it leads to reduced levels of both, frataxin mRNA and protein. Among other functions, frataxin a small mitochondrial protein, is also involved in assembly of iron-sulfur-cluster (FeS) proteins. As there are several isoforms of Frataxin and their individual exact physiological role is unknown we wanted to develop a readout for frataxin´s ultimate function, namely the formation of FeS-clusters. For this purpose, a dynamic protein interaction system based on protein fragment complementation was established. Human glutaredoxin 2 (GRX2), a protein that is only able to dimerize by binding 2Fe2S clusters, has been fused to two renilla luciferase fragments that can only complement each other when brought into close proximity. Thus FeS-cluster dependent dimerization of GRX2 can be measured via the bioluminescence of complemented luciferase. As a negative control the C37A-mutant of GRX2, that is no longer able to coordinate a metallocluster has been chosen. With this assay it will be possible to measure whether small molecules or hormons, like erythropoietin have an influence on the frataxin-dependent formation of FeS-clusters and it could thus be a useful tool to screen for novel drugs to combat Friedreich´s Ataxia.

Sandra Pfurtscheller[1], A. Eigentler[2], S. Bösch[2], R. Schneider[1]

[1]Institute of Biochemistry, Center for Molecular Biosciences CMBI, University of Innsbruck, Innsbruck, Austria
[2]Department of Neurology, Medical University of Innsbruck, Innsbruck, Austria

sandra.pfurtscheller@uibk.ac.at

Class 1-type histone deacetylases in filamentous fungi: Essential enzymes with fungal specific properties

Angelo PIDRONI[1], I. BAUER[1], S. VERGEINER[1], S. GROSS[1], G. BROSCH[1], M. HERMANN[2], S. GRAESSLE[1]

[1]Division of Molecular Biology, Innsbruck Medical University
[2]Department of Anesthesiology and Critical Care Medicine, Innsbruck Medical University

angelo.pidroni@i-med.ac.at

The rapid increase of invasive fungal infections and growing resistance of fungal pathogens to conventional antifungal therapies result in high mortality rates that demand novel strategies to combat systemic mycosis. Due to adverse effects of many of the antifungal substances known, toleration by patients is a major challenge in the discovery of new therapeutic approaches. Detailed knowledge of fungal-specific attributes supporting virulence, germination, invasion, dissemination or drug-resistance is a prerequisite to define novel targets for efficient antifungal drugs with no or only moderate side effects.
In recent years, it has become more and more apparent that histone deacetylases (HDACs) play a decisive role in the regulation of genes involved in fungal growth, sporulation, pathogenicity, and production of important secondary metabolites. Moreover, one of these enzymes, the class 1 HDAC RpdA, was identified to be even crucial for growth and development of Aspergillus nidulans1. Analysis of strains expressing different mutated RpdA variants revealed, that a fungal specific, C-terminal region is required for the biological function of the enzyme. Since RpdA, like most classical HDACs, functions as part of large protein complexes within the nucleus, we expressed tagged RpdA variants in order to analyze their cellular location and catalytic activity.
Interestingly our investigations clearly demonstrate, that a few charged residues within and adjacent to the essential fugal specific motif are required for both, nuclear targeting and catalytic activity of RpdA and thus cannot be deleted or substituted without leading to an atrophic phenotype with a drastic restriction in radial growth of the mutant strains.
Considering these results, RpdA with its fungal specific motif represents a promising target for novel HDAC-inhibitors that might, in addition to their growing significance as anti-cancer drugs, become important in the therapy of invasive fungal infections of immuno-compromised patients apart or in combination with drugs, administered within classical antifungal therapy regimes.

Nanogel-Mediated Protein Replacement Therapy for Autosomal Recessive Congenital Ichthyosis (ARCI)

Roswitha Plank

Innsbruck Medical University, Center for Dermatogenetics, Div. of Human Genetics, Austria

roswitha.plank@i-med.ac.at

The development of new therapies for rare diseases is often neglected by pharmaceutical companies since there is little profit to be made in this field. For affected patients however, new, effective treatments could greatly improve their quality of life. Moreover, given that there are more than 8,000 diseases that meet the criteria, rare diseases as a whole are frequent and affect many patients. Autosomal recessive congenital ichthyosis (ARCI) is a rare, monogenic cornification disorder with varying degrees of erythema, epidermal scaling and impaired skin barrier function, leading to transepidermal water loss and frequent infections. To date, most treatment schemes offer only symptomatic relief. Causes of ARCI include a number of genetic mutations, predominantly in genes involved in epidermal differentiation. About 30% of all patients have mutations in TGM1, which codes for the enzyme transglutaminase 1 (TGase1). In this study we aim to develop a novel, personalized therapy for ARCI patients with TGM1 mutations by substituting the defective protein TGase1.
We have generated 3D full thickness skin models with keratinocytes and fibroblasts extracted from control persons as well as TGM1 patients as models for therapeutic testing. The models were comprehensively characterized for gene expression and synthesis of different epidermal markers by immunohistochemistry, real time PCR analysis and Western blot analysis. Our full thickness skin models develop a stratum corneum with a functional barrier comparable to the epidermal barrier of the skin, as assessed by Franz-cell analysis. As expected, controls showed a clear difference in barrier activity compared to TGM1-patient or TGM1-knockdown (KD) 3D skin models, justifying their use as model system to test our novel therapeutic approach.
In order to deliver replacement TGase1 into the keratinocytes of the skin model we encapsulated the enzyme within thermoresponsive PNIPAM-dPG nanogels (NG), which were then applied in varying concentrations and treatment schedules onto the skin models. Interestingly, after application of the TGase1/NG-complex on TGM1-patient and TGM1-KD models, we observed immunohistochemical staining for TGase1 and greatly improved barrier function compared to the untreated models. This effects could also be shown in a dose dependent manner: The more TGase1 was loaded onto the NG the more the barrier improved. Specific activity tests for TGase1 also determined increased enzyme activity in the treated TGM1-KD models. Since it has been shown that reduced levels of TGase1 activity are sufficient to prevent the manifestation of the diseased skin phenotype, the data here suggests that externally applied TGase1 both enters into keratinocytes and successfully replaces its natural function in TGM1 -KD or mutant skin models.
MTT cytotoxicity assays have indicated high biocompatibility of the PNIPAM-dPG nanogel. Further experiments are still needed to determine the best TGase1 dose and the fate of the nanogel following application to skin.
Our findings demonstrate an advanced topical drug delivery system suitable for cutaneous protein replacement as a promising approach towards personalized, causative therapy for ARCI. After further experiments with patient cells, optimization of epidermal delivery and toxicity tests we will adapt our system for other proteins involved in congenital ichthyosis such as the lipoxygenases 12R-LOX and eLOX-3.

The role of the multivesicular body pathway in sphingolipid homeostasis

Oliver Schmidt[1], Verena Baumann[1], Mihaela Angelova[2], Ingrid Stoffel-Studer[3], Robbie Loewith[4], Zlatko Trajanoski[2], Matthias Peter[3], David Teis[1]

[1]Div. of Cell Biology, Biocenter, Medical University of Innsbruck, Austria
[2]Div. of Bioinformatics, Biocenter, Medical University of Innsbruck, Austria
[3]Institute for Biochemistry, ETH Zurich, Switzerland
[4]Department of Molecular Biology, University of Geneva, Switzerland

oliver.schmidt@i-med.ac.at

The multivesicular body (MVB) pathway mediates the selective degradation of ubiquitinated membrane proteins in lysosomes and therefore has a prominent role in downregulating mitogenic signaling. Cargo sorting and the biogenesis of MVBs are mediated by the endosomal sorting complexes required for transport (ESCRTs). Despite the prominent role of the MVB pathway in down-regulating signaling from growth factor receptors, it is unclear how the ESCRT-dependent degradation of the membrane proteome contributes to basic cellular homeostasis.
To systematically address the role of the ESCRT machinery in cellular homeostasis we have used genome wide synthetic genetic array analysis in Saccharomyces cerevisiae. We identified 118 genes that are required for the growth of an ESCRT deletion strain (vps4Δ). These genes are necessary for the survival of ESCRT mutants and thus help to cope with intracellular membrane protein accumulations.
Among them, we isolated several genes involved in positive regulation of sphingolipid metabolism. Consistently ESCRT mutants show altered sphingolipid metabolism and trafficking. The role of the MVB pathway on sphingolipid metabolism and its potential implications on cellular homeostasis and pathologies will be discussed.

Silvering and parasitism affect ROS defense capability in swimbladder gas gland cells of the European eel (*Anguilla anguilla*)

Gabriel Schneebauer, Bernd Pelster

gabriel.schneebauer@uibk.ac.at

In a process called silvering, European eels prepare for their long-distance migration from European fresh water systems to the Sargasso Sea for reproduction. During this journey, eels perform extended diel vertical migrations, and the concomitant changes in hydrostatic pressure significantly affect the swimbladder, functioning as a buoyancy organ. As the swimbladder is primarily filled with oxygen, the constituting gas gland cells have to cope with extreme hyperoxic conditions, which typically are accompanied by the generation of reactive oxygen species (ROS), and result in harmful oxidative stress. In addition, since the introduction of the parasitic nematode Anguillicola crassus in the early 1980's, swimbladder function of most of the European eels is impaired by the infection with this parasite. However, the exact ROS detoxification pathways in gas gland cells and the effects of silvering and infection on these pathways are still unknown. We analyzed muscle and swimbladder tissue of uninfected yellow, infected yellow, uninfected silver and infected silver eels for the activities of superoxide dismutase (SOD), catalase (Cat), glutathione peroxidase (GPx), and glutathione reductase (GR), the antioxidant glutathione (GSH+GSSG), and additionally the oxidative-stress marker malondialdehyde (MDA). In swimbladder gas gland cells, we found higher concentrations of GSH+GSSG and higher activities of SOD, GPx and GR, as compared with muscle tissue, thus suggesting SOD and the glutathione cycle to be of high importance for ROS detoxification. Comparing uninfected yellow with uninfected silver eels, the concentration of GSH+GSSG and the activity of SOD were higher after silvering, corresponding with lower levels of oxidative stress. Whereas in yellow eels the infection with A. crassus had no effect, in silver eels the gas gland cells' capacity to cope with ROS was significantly impaired and resulted in increased oxidative stress.

Understanding the molecular basis of cell death triggered by inhibition of Check-point kinase 1

Fabian Schuler[1], Sehar Afreen[2], Miriam Erlacher[2], Andreas Villunger[1]

[1]Department of Developmental Immunology, Medical University Innsbruck
[2]Department of Pediatrics and Adolescent Medicine, University Medical Center, Freiburg

fabian.schuler@i-med.ac.at

Chk1 is an essential gene in cell cycle control and DNA-damage response, amongst others, regulating Cdc25 phosphatases to interfere with cell cycle progression. Knocking-out Chk1 in mice results in blastocyst death due to G2/M-failure. In our own work we could show that cell death inflicted by loss of Chk1 cannot be prevented by concomitant loss of p53 and/or Caspase-2. Conditional knockout in the hematopoietic system resulted in impaired hematopoiesis and a loss of colony formation capacity of LSK-cells (Hematopoietic stem and progenitor cells). Concomitant Bcl-2 overexpression or loss of p53 doesn't rescue this phenotype pointing at no involvement of classical apoptotic cell death as a consequence of premature cell cycle entry, at least of hematopoietic cells. As Chk1-inhibitors are developed for tumor therapy, we also tested their effects on cord-blood derived CD34+ human hematopoietic stem cells that stop proliferating when treated with PF-477736, a highly specific Chk1 inhibitor, and subsequently die by an unknown mechanism often termed as 'mitotic catastrophe'. Similar results could be obtained using murine Hox-B8 immortalized neutrophil progenitor cells that we will use as a tool for screening the genetic basis of this type of cell death. Understanding the mechanism of cell death triggered by Chk1-inhibition will help to understand possible side effects when used to treat cancer.

Structural and functional analysis of CDK16

Simon Spiegl, Elisabeth Pfeiffenberger, Petra Mikolcevic, Stephan Geley

Division for Molecular Pathophysiology, Biocenter, Innsbruck Medical University

simon.spiegl@i-med.ac.at

Among the family of cyclin dependent kinases (CDKs), PCTAIRE (PCTK) AND PFTAIRE kinases (PFTK) are still poorly understood. Their high structural similarity, common activators (Cyclin Y (CCNY)/Cyclin Y-Like 1 (CCNY-L1)) and overlapping expression patterns suggest a high degree of redundancy. Recent findings, concerning their regulation as well as data derived from genetically modified organisms point to specific functions that might be important for tumourigenesis. Moreover, aberrant expression and mutation of these kinases have also been found in tumours. Consitutive active Wnt signalling is a hallmark of several cancer subtypes, particularly colon carcinoma in which driving mutations in ß-catenin and APC genes are frequently found. In other tumors, e.g. in mammary carcinoma, such mutations are rare, but WNT signalling is kept high due to autocrine signalling. By regulating the WNT co-receptor LRP5/6, CCNY-activated kinases regulate the responsiveness of the WNT target cell and could therefore contribute to canonical Wnt signalling not only in mammary but also in various other tumours where downstream mutations are not sufficient to drive tumour growth. Despite the evidence that these kinases have essential physiological functions and may contribute to a major pathway in various tumours, very little is known about their expression and regulation. To investigate the role of CCNY-activated kinases in WNT signalling I have established various tumor cell lines and used a dominant negative CDK16 version to analyze the impact of this group of CDKs. Further I am now trying to define the specific CDKs involved in this pathway and explore their impact on proliferation and cell survival. In addition, crystallization trials have been started in order to obtain the structure of the CDK16/CCNY complex, which would help to understand the mechanism of activation by CCNY. This will be important for understanding the mode of action of CCNY-dependend kinases and for the design of novel inhibitors.

First steps towards understanding ESCRT mediated membrane remodeling

Simon Sprenger[1], Sabine Weys[1], Manuel Alonso Y Adell[1], Klaus Faserl[2], Herbert Lindner[2], David Teis[1]

[1]Division of cell biology, Medical University Innsbruck
[2]Division of clinical biochemistry, Medical University Innsbruck

s.sprenger@i-med.ac.at

The ESCRT (endosomal sorting complexes required for transport) machinery plays an important role in the selective degradation of membrane proteins via the multivesicular body pathway (MVB) in lysosomes and is involved in in cytokinesis, HIV budding and nuclear envelope closure. A common denominator for the last step in these processes is the assembly of spiral ESCRT-III polymers and the function of the AAA ATPase Vps4.
We have shown that the coordinated binding of Vps4 to the ESCRT-III subunits Snf7 and Vps2 is required for ESCRT-III disassembly and membrane budding process. How ESCRT-III and Vps4 function together to remodel membranes in a fundamental yet poorly understood cell biological process.
We hypothesize that the Vps4 induces conformational changes in the ESCRT-III filament to convert an initial membrane deformation complex into a membrane scission machine. The goal is to understand how Vps4 remodels the ESCRT-III complex during ILV formation.
By using yeast as the best-suited model organism, we will combine immunoprecipitation and cross-linking mass spectrometry identify conformational changes in the ESCRT machinery, and how they drive membrane remodeling and scission. Additionally we will use purified ESCRT subunit to reconstitute the ESCRT machinery on artificial liposomes to further study the assembly and disassembly in vitro. Ultimately, we hope that these experiments will provide better insight into the molecular mechanisms required for ESCRT-III/Vps4 mediated membrane budding . First results will be presented.

Skeletal Muscle Derived Multipotent Mesenchymal Stromal Cells for Smooth Muscle Regeneration

Marco Thurner, Faheem Asim, Eva Margreiter, Angelika Flörl, Kristian Pfaller, Esther Schamschula, Jakob Troppmair and Rainer Marksteiner

Innovacell Biotechnologie AG

The in vitro and in vivo multipotent differentiation capacity of Mesenchymal Stem Cells (MSCs) is well known. Although MSCs were originally isolated from adult bone marrow, it has been found that they are located in virtually all postnatal organs and tissues including brain, spleen, liver, kidney, lung, bone marrow, muscle, thymus and pancreas. Their ubiquitous presence may be explained by the hypothesis that they commonly origin from perivascular cells. Since during the last decades, the term MSC was used for cells of mesenchymal origin without proof of stemness, the International Society for Cellular Therapy (ISCT) proposed to use the term Multipotent Mesenchymal Stromal Cells (MSCs) instead. Furthermore, the society suggested using common criteria to identify MSCs independently of the tissue of origin or stemness. These criteria include plastic adherence of the isolated cells, expression of cell surface markers CD105, CD73 and CD90, lack of cell surface markers CD14, CD45, CD19 and HLA-DR, as well as multilineage potential for differentiation towards adipogenic-, osteogenic- and chondrogenic lineages in vitro.
In the adult skeletal muscle two distinct types of adult stem/progenitor cells can be isolated: On the one hand to myogenic differentiation committed satellite cells, which are located beneath the basal membrane of the muscle fiber, which give rise to myoblasts and are able to regenerate skeletal muscle; on the other hand multipotent mesenchymal stromal cells, which might harbor high potential for use in regenerative medicine and cellular therapy. Previous work by others suggests that MSCs harbor the potential to differentiate to the smooth muscle cell lineage.
Smooth muscle tissue consists of highly differentiated, single-nucleated cells, which are electrically coupled and innervated primarily by the autonomous nervous system. Smooth muscle tissue is found throughout the human body at sites such as the walls of blood vessels (vascular smooth muscle) within the tunica media of arteries, arterioles and veins, as well as the urinary (i.e. urinary smooth muscle of the internal sphincter) and gastrointestinal tract (rectal internal sphincter). The lack of proper medication and treatment for diseases resulting from smooth muscle tissue dysfunction, such as overactive bladder, urinary- and fecal incontinence warrants the cell therapy and tissue engineering based approaches.
In the present study we established protocols for the isolation and characterization of multipotent mesenchymal stromal cells (MSCs) from human skeletal muscle tissue. We studied the in vitro differentiation of skeletal muscle-derived MSCs to smooth muscle cells. Furthermore, we established to use a 3D cell culture model to fabricate rings from skeletal muscle derived MSCs, which are currently further evaluated for their use in the replacement of dysfunctional internal sphincter in urinary- or fecal incontinence in vivo.

Investigating the Role of the Orphan G-protein Coupled Receptor Gpr161 in Hedgehog Signalling

Philipp M Tschaikner[1], Verena Bachmann[2], Eduard Stefan[2], Pia Aanstad[1]

[1]Institute of Molecular Biology, University of Innsbruck, Center for Molecular Biosciences Innsbruck
[2]Institute of Biochemistry, University of Innsbruck, Center for Chemistry and Biomedicine

philipp.tschaikner@uibk.ac.at

The Hedgehog (Hh) signalling pathway plays essential roles in cell fate specification, cell proliferation, stem cell homeostasis, and has also been linked to other cell biological processes such as axon guidance and cell migration. While the Hh pathway has been studied extensively in the last thirty years, there are still many open questions in the understanding of how this pathway is regulated downstream of ligand-binding to the Hh receptor Patched (Ptc) and how tissue- and cell type-specific outcomes can arise.
Protein kinase A (PKA) is a central negative regulator of Hh signalling, but how PKA activity is switched off upon Hh pathway activation has remained a mystery. Moreover, although Hh signal transduction takes place within the primary cilium, the exact subcellular localisation where PKA regulation ocurs is unclear. Recently it was shown that the orphan G-protein coupled receptor Gpr161, a negative regulator of Hh signalling, localises to the primary cilium and activates PKA, and exits the cilium upon Hh pathway activation. Our preliminary results have shown that Gpr161 is both a target of PKA and that it acts to localise PKA activity, suggesting that Gpr161 plays a central role in the regulation of PKA activity downstream of Hh signalling.
The aim of my project is to elucidate the roles of Gpr161 and how it acts on Hh signalling on a molecular level, using zebrafish as an in vivo vertebrate model organism in combination with different mammalian cell culture systems.

Interactomes of the LAMTOR complex: Merging Point of Different Signaling Pathways on Late Endosomes and the Identification of C10orf32

Teodor Yordanov[1], Taras Stasyk[1], Michael Hess[2], Lukas A. Huber[1]

[1]Medical University Innsbruck, Biocenter, Section of Cell Biology
[2]Medical University Innsbruck, Department of Anatomy, Histology and Embriology

teodor.yordanov@i-med.ac.at

Fundamental cellular functions such as proliferation, differentiation, migration and apoptosis require the precisely coordinated action of a number of proteins in macromolecular assemblies. These provide spatial and temporal specificity to a signal, which needs to trigger a specific defined response at a designated location. The Late endosomal/lysosomal Adaptor and MAPK and mTOR activator (LAMTOR) complex facilitates MAP kinase signal transduction and is also essential for mTORC1 kinase signaling and amino acid sensing on this specific subcellular location.
In a Tandem Affinity Purification coupled to mass spectrometry (TAP-MS) approach, novel LAMTOR complex- interacting proteins were identified. Some of these belong to well-known and characterized group of proteins, such as the Biogenesis of Lysosome-related Organelle Complex 1 (BLOC-1). This is involved in the regulation of maturation of lysosomes and lysosome-related organelles like melanosomes and platelet dense granules and is affected in endosomal diseases, such has Hermansky- Pudlak syndrome. Yet others, such as C10orf32, Snapin, C17orf59 or LOH12CR1 are only surfacing in the research focus of the scientific community. Therefore in my study I have concentrated my effort on pinpointing the biological function of the largely unknown protein C10orf32 in the context of vesicle/cargo trafficking, endosomal biogenesis and signaling.
The major tools involved in the project include the generation of two Knock Out (KO) cell lines by CRISPR/Cas9 genome editing; i.e. HeLa cells and the fibrocarcoma cell line HT1080. While the former is a well-established cell line to study broad ranges of defects in endocytosis, trafficking and signaling, the latter one is a N-Ras activated sarcoma cell line, which, as I have shown, is able to spontaneously build enlarged endosomes. Upon analysis of this specialized cell line, using indirect immunofluorescence, electron microscopy of cryofixed samples and live-cell imaging I have been able to show that this process is greatly altered in C10orf32 KO cell lines, as those retain small endosomal vesicles. Interestingly we have also shown that C10orf32 interacts with a number of SNAREs, some of which are involved in the homotypic fusion of late endosomes. Therefore, it is tempting to speculate that C10orf32 is involved in the maturation and fusion of endosomes.

Bioadhesion in ascidian

Fan Zeng, Julia Wunderer, Peter Ladurner, Willi Salvenmoser, Ute Rothbächer

Zoological Institute, University Innsbruck, Austria

fan.zeng@uibk.ac.at

Marine bio-adhesion research has increasingly inspired the design of bio-mimetics for tissue compatible glues in the medical field and antifouling compounds for shipping and aquaculture. Tunicates (ascidians) are not only important, because of their phylogenetic position, being the sister group to vertebrates, but also ecologically they are one of the major biofoulers massively populating artificial surfaces like ship hulls causing serious damage. In recent years it therefore became of great interest to understand how bio-adhesion is functioning in ascidians. In the host lab we use the ascidian Ciona intestinalis as model organism to study the adhesive properties of their larvae. Recent research demonstrated that larval adhesion organs (papillae) of Ciona intestinalis mainly consist of glue secreting collocytes, papillary neurons and supporting cells. However, detailed structure of cellular components of papillae and neurons, the exact secretion mechanism and the involved molecules are not fully described and need further investigation. Consequently, we aim at visualizing papillary cellular components by fluorescent labelling of neurons accompanied by immune-histochemical staining. Furthermore, we analyse candidate genes that might be involved in papillae organogenesis. Finally, we plan to biochemically analyse (Protein pulldown, Mass-spectrometry) candidate adhesion proteins and required post-translational modification such as glycosylation.

Elevated fibrinogen levels in thrombocytopenic sepsis patients

Background: Thrombocytopenia is a frequent comorbidity in intensive care unit (ICU) patients and often caused by sepsis. Non-resolution of thrombocytopenia (in adult, septic patients) was shown to be associated with 28-day mortality. Usually, fibrinogen levels are increased during sepsis. We analyzed the effects of elevated fibrinogen levels on survival in thrombocytopenia in ICU-patients with sepsis. We hypothesized that higher fibrinogen levels in the course of sepsis would be associated with survival and could be beneficial especially for thrombocytopenic patients.
Methods: Data from 915 sepsis patients, admitted to the neurological, surgical, traumatological or general intensive care unit of the Medical University of Innsbruck between 2000 and 2014 were collected retrospectively at the day of the peak level of C-reactive protein (CRP) as well as 3 days before until 3 days after that peak. Patients were divided into survivor/nonsurvivor, thrombocytopenic (TCP, platelet count <150 G/L)/non-thrombocytopenic (non-TCP, platelet count >150 G/L) and high fibrinogen levels (HF, fibrinogen plasma level >400 mg/dl)/ normal or low fibrinogen levels (LF, fibrinogen plasma level <400 mg/dl), respectively.
Results: Of all patients, 46 % suffered from TCP. Patients in the TCP group have a significantly higher proportion of nonsurvivors than the non-TCP group ($p<0.001$). Regarding the non-TCP group, no significant differences are detected between the HF and the LF group in survival, whereas in the TCP group there are significantly more survivors in patients with high fibrinogen levels ($p<0.001$). Moreover, all patients in the HF group (independently of TCP) present a higher proportion of survivors than the LF group ($p<0.001$).
Conclusions: About half of all sepsis patients develop TCP what is associated with a worse outcome. Septic TCP patients with high fibrinogen levels have a higher survival rate and lower bleeding events. Therefore, fibrinogen might compensate for a low platelet count in septic patients in regards of survival and bleeding events.

Miriam Bachler, D. Fries, B. Schenk

Department of General and Surgical Critical Care Medicine

mirjam.bachler@tirol-kliniken.at

Interaction of *Pseudomonas aeruginosa* with complement

Daniela Bichler, Z. Banki, D. Orth-Höller, R. Würzner, H. Stoiber

Dept. of Hygiene, Microbiology and Social Medicine, Innsbruck Medical University

daniela.bichler@i-med.ac.at

Background: According to the Infectious Disease Society of America, Pseudomonas aeruginosa is among the top-priority dangerous drug-resistant microbes. Presently, no vaccination is available. Thus alternative approaches to combat this microbe are urgently needed. Among the numerous immune and inflammatory responses upon acute infection of the lung, experiments indicate that the complement system plays a critical role in the protection against P. aeruginosa. However this complement-mediated involvement in the immune response does not result in a complete elimination of the microbe, as P. aeruginosa acquires and binds fH, a regulator of complement activation (RCA) in fluid phase. By binding of fH, P. aeruginosa interferes with the activation pathways of the complement system and block the induction of the lytic pathway. Therefore, P. aeruginosa is protected against complement-mediated lysis (CML). We aim to inhibit the binding of fH to P. aeruginosa that should result in the efficient inductionof CML, both in vitro and in vivo.
Methods: To investigate our aim, we will analyze the binding sites of fH to the microbe by ELISA and FACS utilizing fH-derived sequences. The bactericidal effect of these fH-derived peptides will be tested by in vitro lysis assays using normal human serum (NHS) as a source of complement.
Conclusion: Based on these results we can conclude that fH-derived peptides may provide a therapeutic means to enhance the immune response against P. aeruginosa.

The Viral Vector Vaccine VSV-GP as HIV-Vaccine Platform

Background: Our group has recently shown that VSV pseudotyped with the glycoprotein (GP) of the lymphocytic choriomeningitis virus, VSV-GP, is a potent vaccine vector, overcoming limitations of wild type VSV.
Objective: Here, we evaluated the potential of VSV-GP as a vaccine vector for infectious disease such as HIV.
Methods: We incorporated antigens from pathogens, e.g. different variants of HIV envelope, or marker genes into the genome of VSV-GP and generated infectious viruses via reverse genetics. These viruses were analyzed in vitro for antigen expression, location and conformation. After mouse immunization studies distribution and kinetics of infected cells and antigen-specific as well as vector-specific immune responses were analyzed.
Results: Infectious viruses containing antigens from HIV or marker genes such as luciferase were generated. HIV envelope variants were expressed in VSV-GP infected cells and incorporated into VSV-GP particles. Crucial epitopes for the induction of neutralizing antibodies against HIV such as MPER, CD4 binding side and V1V2 loop were present on the surface of VSV-GP-env particles. The addition of an extra gene did not attenuate VSV-GP replication. After intramuscular immunization, viral replication was limited to injection side and the draining lymph nodes. No neutralizing antibodies against VSV-GP were induced even after seven boost immunizations. Therefore, homologous boost immunization was highly efficient and high titers of HIV-specific antibodies were induced.
Conclusion: Taken together, VSV-GP is non-neurotoxic, induces potent immune responses, enables boosting and thus is a promising novel vaccine vector platform.

Anika Bresk[1], J. Kimpel[1], R. Tober[1], M. Krismer[1], T. Hofer[1], HX. Liao[3], BF. Haynes[3], Z. Banki[1], L. Egerer[1],D. von Laer[1]

[1]Division of Virology, Medical University of Innsbruck, Innsbruck, Austria
[2]Fraunhofer Institute for Cell Therapy and Immunology, Leipzig, Germany
[3]Duke Human Vaccine Institute, Durham, NC; Department of Medicine, Duke University School of Medicine, Durham, NC, US

anika.bresk@i-med.ac.at

Relevance of platelets and complement for the pathogenesis of invasive fungal infections

Hemalata Deshmukh[1], G. Rambach[1], M. Hagleitner[1], M. Hermann[2], C. Lass-Flörl[1], C. Speth[1]

[1]Division of Hygiene and Medical Microbiology, Innsbruck, Austria
[2]Department of Anaesthesiology and Critical Care Medicine, Innsbruck, Austria

hemalata.deshmukh@i-med.ac.at

Background: Aspergillus and mucormycetes species are leading causes for invasive fungal infections. To better understand the antifungal immune reaction and relevant pathomechanisms we studied the interaction of the fungi with platelets and the complement system, two important elements of the innate immunity. We hypothesized that Aspergillus fumigatus secretes soluble galactosaminogalactan (GAG) which induces platelet activation and complement deposition on their surface. We expanded these studies to other Aspergillus species as well as to different mucormycetes.
Methods: Clinical isolates of different Aspergillus and mucormycete species were grown in RPMI medium for 48 hours followed by filtration to obtain the fungal supernatants (SN). Activation and complement deposition on platelets derived from healthy donors were investigated by FACS analysis using specific antibodies. GAG production was detected on the hyphae of Aspergillus and mucormycete spp. as stained with FITC-labeled SBA lectin.
Results: The culture supernatants of all tested clinical isolates of Aspergillus fumigatus and Aspergillus flavus triggered significant platelet activation as measured by quantification of the activation marker CD62P. In parallel, SN-induced deposition of complement factor C3 on the platelet surface as well as formation of the terminal complement complex (TCC) could be demonstrated. However, no or only weak induction of platelet activation or complement deposition on their surface could be achieved using the culture supernatants derived from A. niger, A. versicolor or A. terreus as well as from all mucormycetes species. These results correlated perfectly with the expression of GAG by the different fungi, as demonstrated by immunofluorescence/confocal microscopy using specific staining with SBA lectin.
Conclusion: The correlation between GAG expression by the Aspergillus and mucormycete species and isolates with the capacity of the fungal supernatants to stimulate platelet activation and opsonisation underlines our hypothesis that the polysaccharide GAG might be an important fungal immunomodulatory molecule. Putative consequences of its activity are platelet-mediated antifungal attack but also the formation of platelet thrombi.

The catecholamine dopamine regulates iron homeostasis

Stefanie Dichtl, David Haschka, Egon Demetz, Manfred Nairz, Malte Aßhoff, Markus Seifert, Sylvia Berger, Günter Weiss

Department of Internal Medicine VI, Medical University of Innsbruck, Austria

stefanie.dichtl@i-med.ac.at

Objective: Siderophores are catechol based compounds which can bind iron. Iron is an essential growth factor for mammalian cells and microbes. Based on previous observations, showing increased bacterial growth in the presence of catechols, we asked whether this may be referred to hormone mediated alterations of iron homeostasis even in a non-infectious way.
Material and methods: We studied the effects of the catecholamine dopamine on the regulation of iron in bone marrow derived macrophages obtained from C57Bl/6 wild-type mice and littermates knocked out for lipocalin-2, a mammalian siderophore binding peptide.
Results: Administration of dopamine to macrophages resulted in a dose dependent increase of heme oxygenase-1 and ferroportin expression, the latter being the major cellular iron exporter. The upregulation of the ferroportin expression is a consequence of the increased intramacrophage iron concentration, which results in an augmented ferritin expression. These effects were independent from the presence/ absence of the siderophore binding peptide lipocalin-2. Experiments with tyrosine, a precursor of catecholamines without the 1,2-dihydroxybenzene group, doesn't show comparable results.
Conclusion: Our data demonstrate that dopamine can modulate iron homeostasis in macrophages. This catecholamine is able to increase the intracellular iron. Therefore dopamine is able to act as a mammalian siderophore or can chelate iron directly.

Identification and characterization of antifungal drug targets in *Aspergillus fumigatus*

Anna-Maria Dietl[1], J. Amich[2], S. Leal[3], U. Binder[4], S. Krappmann[5], N. Osherov[6], T. Dandekar[7], H. Haas[1]

[1]Dept. of Molecular Biology, Medical University of Innsbruck
[2]IZKF Forschergruppe für Experimentelle Stammzelltransplantation, Würzburg
[3]Dept. of Ophthalmology, Case Western Reserve University, Cleveland, Ohio
[4]Dept. of Hygiene & Medical Microbiology, Medical University of Innsbruck
[5]Dept. of Clinical Microbiology, Immunology and Hygiene, University Hospital Erlangen
[6]Dept. of Human Microbiology, Sackler School of Medicine, Tel Aviv
[7]Dept. of Bioinformatics, University of Würzburg

anna-maria.dietl@i-med.ac.at

Background: *Aspergillus fumigatus* is the most prevalent airborne fungal pathogen causing invasive fungal infections in immunosuppressed individuals. Limitations in antifungal therapy arise from non- specific symptoms of infection, poor diagnostics and comparatively few options for treatment. The aim of this study is to explore the metabolism of *A. fumigatus* on a comprehensive scale as essential virulence determinant to generate a collection of *A. fumigatus* strains with a focus on primary metabolism to target fungal pathways that are absent in mammals.
Methods: Based on the annotated genome of *A. fumigatus*, metabolic network reconstruction served to identify fungal-specific pathways and key reactions. Predictions for unique enzymes resulted in a candidate list of genes, the inactivation of which is likely to result in an auxotrophic phenotype. The virulence potential of the generated auxotrophic mutant strains was then analysed in various host niches.
Results: We identified four essential biosynthetic pathways in *A. fumigatus*: (i) biosynthesis of the amino acid histidine, (ii) biosynthesis of the co-enzyme siroheme, which is essential for sulfate and nitrate assimilation, (iii) biosynthesis of the vitamin riboflavin and (iiii) biosynthesis of the vitamin pantothenic acid. The loss of histidine biosynthesis resulted in attenuation of pathogenicity in four virulence models: murine pulmonary infection, murine systemic infection, murine corneal infection, and wax moth larvae *Galleria mellonella*. Defective siroheme biosynthesis led to mild attenuation in the *Galleria mellonella* infection model but did not affect pathogenicity in murine infection models. Moreover, preliminary data indicate that inactivation of biosynthesis of riboflavin or pantothenic acid resulted in attenuated virulence of *A. fumigatus* in murine pulmonary infection models.
Conclusion: Via generation of auxotrophic mutant strains, genes encoding components of biosynthetic pathways for histidine, siroheme, riboflavin and pantothenic acid were identified and analysed by mutant strain phenotyping and virulence testing. The results characterize the host niche and reveal targets for development of novel antifungal therapeutic approaches.

Recall responses to tetanus and diphtheria vaccination: A comparison of young and elderly adults

Marco Grasse[1], Andreas Meryk[1], Michael Schirmer[2], Beatrix Grubeck-Loebenstein[1], Birgit Weinberger[1]

[1]Div. of Immunology, Institute for Biomedical Aging Research, Universität Innsbruck
[2]Department of Internal Medicine VI, Medical University Innsbruck

marco.grasse@uibk.ac.at

Immunization is one of the most successful health intervention against infectious diseases. However, the efficacy of vaccination is reduced in old age. Our study analyzed specific immune responses following a booster vaccination containing tetanus and diphtheria toxoid in healthy elderly (>60y; n=87) and young volunteers (25-40y; n=46). Long term protection was evaluated for 27 elderly and 17 young adults 5 years later. In addition, antigen-specific T-cells producing a panel of cytokines were quantified.
Before the vaccination 9% of the older and none of the young individuals had tetanus-specific antibody levels below the protective limit. The booster induced sufficient protection in both age groups for the following 5 years. The protection against diphtheria was almost equal before the vaccination (52% for the elderly and 48% for the young donors). Antibody concentrations increased significantly 4 weeks after vaccination, but dropped substantially over 5 years leaving again 54% (elderly) and 24% (young) below protective antibody levels. Thus, compared to the elderly young adults have a significantly better, but still insufficient maintenance of diphtheria-specific antibodies. We found correlations between diphtheria-specific antibodies and diphtheria-specific T-cells producing different cytokines. Among those were GM-CSF-producing T-cells which we detected in a lower frequency in elderly compared to young adults.
In conclusion, our findings demonstrate that a booster vaccination induce long-lasting immunity against tetanus but not diphtheria, particularly in elderly people. GM-CSF might be useful as an adjuvant to improve diphtheria vaccination. We set up a mouse model to further investigate this question.

The *Candida albicans* factor H binding molecule Hgt1p – in vitro and in vivo evidence that it functions as virulence factor

Samyr Kenno, Dorothea Orth-Höller, Reinhard Würzner

Division of Hygiene & Med. Microbiology, Innsbruck Med. University, Innsbruck

samyr.kenno@i-med.ac.at

Background: The complement system is tightly controlled by several regulators. In particular Factor H (FH) is preferentially acquired by pathogens conveying resistance to complement attack.
Objectives: The aim of the study was to determine whether the FH binding molecule „high affinity glucose transporter 1" (CaHgt1p) of *Candida albicans* is a potential virulence factor.
Methods: An in vitro phagocytosis study was performed to demonstrate the ability of *C. albicans* to bind FH and avoid the phagocytosis. The assay was performed using *C. albicans* wild type (SN-152) and knock-out strain (hgt1Δ/Δ). Both strains were opsonized with human serum (HS) and stained with fluorescein isothiocyanate (FITC). Fresh PMNs cells were co-cultured with both strains and positive PMNs were detected using FACS analysis.
This is complemented by an in vivo study employing the *Galleria mellonella* model.
Results: The wild type strain, able to bind FH, showed a reduced phagocytosis by PMNs in contrast to hgt1Δ/Δ knock-out strain unable to bind FH.
Galleria larvae inoculated with hgt1Δ/Δ strain lived longer than those inoculated with the wild type strain.
Conclusions: CaHgt1p is not only a complement inhibitor, but also a virulence factor, as corroborated by in vitro and in vivo data.

Bacterial Co-Infection Influences Dendritic Cell Function during Acute HIV-1 Infection

Ulla Knackmuss, W. Posch, M. Steger, P. Chandorkar, M. Blatzer, C. Lass-Flörl, D. Wilflingseder

Department of Hygiene, Microbiology and Social Medicine, Division of Hygiene and Medical Microbiology, Medical University Innsbruck, Innsbruck, Austria

ulla.knackmuss@i-med.ac.at

Background: HIV directly and spontaneously activates complement in semen and at mucosal surfaces and is therefore opsonized with a cloud of covalently bound C3 products in vivo. We earlier illustrated that complement opsonization of HIV-1 and HIV-2 significantly modulates the function and antigen-presenting capacity of immature dendritic cells (iDCs), the first cells to be encountered by HIV-1 at mucosal surfaces. Untreated sexually transmitted infections (STIs) and bacterial vaginosis (BV) are associated with a higher risk of HIV-infection - thus pathogenic bacteria (Gardnerella vaginalis) and microbial products (LPS) might activate DCs upon co-infection and alter their function and antigen-presenting capacity with respect to HIV-C. Thus, during this study we determined modulation of LPS-stimulated DCs using non- (HIV) or C-opsonized HIV-1 (HIV-C).
Methods: We performed binding, fusion, transmission and internalization experiments as well as infection kinetics of differentially opsonized HIV-1 using p24- and IFNg- ELISA, flow cytometry and confocal laser scanning microscopy.
Results: We found similar binding of HIV and HIV-C to iDCs and LPS-DCs, while internalization of both HIV and HIV-C was significantly higher in LPS-DCs. Efficient entry of non- and complement-opsonized virions by fusion was detected in iDCs, while LPS-DCs displayed endocytosis, but not fusion, of HIV and HIV-C. LPS-stimulated DCs were not productively infected by HIV-C, while in iDCs HIV-C caused a significantly higher infection than HIV. Transfer of HIV to CD4+ T cells was significantly higher when iDCs or LPS-DCs were exposed to non-opsonized HIV, therefore pointing to an antiviral effect of DCs loaded with HIV-C independent on the maturation status of the cells. In contrast to that we found a weaker capacity of HIV-C-LPS-DCs to expand and activate specific Cytotoxic T Lymphocytes (CTLs) compared to HIV-C-DCs.
Conclusion: These results indicate an impact of co-infection on the CTL-stimulatory capacity of DCs in presence of pathogenic bacteria or microbial products during acute infection.

Combination of oncolytic virotherapy and DC-based immunotherapy for the treatment of melanoma

Iris Koske[1], Zoltan Banki[1], Isabel Barnstorf[1], Christoph Tripp[2], Patrizia Stoitzner[2], Nikolaus Romani[2], Guido Wollmann[1], Janine Kimpel[1], Dorothee Holm-von Laer[1]

[1]Division of Virology, Innsbruck Medical University, Innsbruck, Austria
[2]University Hospital of Dermatology and Venereology, Innsbruck Medical University, Innsbruck, Austria

iris.koske@i-med.ac.at

VSV-GP, a novel chimeric Vesicular Stomatitis Virus (VSV) pseudotyped with the glycoprotein of the lymphocytic choriomeningitis virus represents a promising oncolytic virus (OV) that preferentially targets and kills cancer cells. Release of tumor antigens and activation of immune response by OV therapy might support dendritic cell (DC)-mediated anti-tumor immunity. Thus in our study we analyzed the efficacy and immune mechanisms of the combination of VSV-GP oncolytic virotherapy with DC-based immunotherapy. Combination of VSV-GP therapy and DC-based vaccination was investigated in the syngeneic subcutaneous B16-OVA melanoma model. SIINFEKL-loaded CpG-activated DCs (DCVacc) and VSV-GP were applied intra- and peritumorally and immune responses were analyzed in the spleen and tumor tissues. The DCVacc/VSV-GP combination therapy resulted in a significantly improved survival compared to single treatments. Surviving mice from the DCVacc/VSV-GP treated group showed a long lasting anti-tumor immunity against B16-OVA and partial anti-tumor immunity against non-OVA B16 melanoma in rechallenge experiments. Analyzing specific cytotoxic T lymphocytes (CTL) responses induced by DCVacc and VSV-GP single and combination treatments we found that both DCVacc and DCVacc/VSV-GP induced comparable levels of OVA-specific CD8+ T cell responses. In addition a strong VSV N peptide-specific CD8+ T cell response was found upon VSV-GP and DCVacc/VSV-GP treatments. The improved therapeutic effect by the DCVacc/VSV-GP combination treatment correlated with increased numbers of tumor infiltrating lymphocytes (TIL) and elevated Tconv/Treg and CD8+/Treg ratios. Furthermore, depletion of CD8+ T cells but not NK cells abrogated the therapeutic effect of DCVacc/VSV-GP. Taken together, the combination of VSV-GP and DC-based immunotherapy might represent a promising therapeutic option for the treatment of melanoma.

Dickkopf-1 (DKK1) and wingless-type MMTV integration site family, member 3A (Wnt3a) in BALB/c and C57BL/6J axial spondyloarthritis models

Kathrin Maly[1], T. de Zordo[2], S. Kemmerling[1], C. Kremser[2], V. Kuhn[2], C. Ammann[1], M. Schirmer[1]

[1]Laboratory of Molecular Cell Biology and Rheumatology, Department of Internal Medicine, Clinic VI
[2]Department of Radiology, Medical University of Innsbruck, Innsbruck, Austria

kathrin.maly@student.i-med.ac.at

Background: The hallmarks of axial spondyloarthritis (axSpA) are inflammation and new bone formation leading to ankylosis of the sacroiliac joint and spine. Little is known about the complex network of inflammatory and osteogenic factors: In brief, bone formation and erosions are associated with wingless (Wnt) proteins and the natural Wnt inhibitor Dickkopf-1 (DKK1), and TNF-α and IL-17 are important pro-inflammatory cytokines in axSpA and negatively associated with osteogenesis in rheumatoid arthritis (RA). In RA, TNF-α induces DKK1 and leads to inhibition of Wnt signaling and joint destruction. In axSpA, TNF-α is also high, but coincides with low DKK1 levels and new bone formation. This study focuses on levels of TNF-α, IL-17A, DKK1 and wnt3a in the late stages of two murine axSpA models with different cytokine responses (BALB/c and C57BL/6J (C57)).
Material and Methods: The spondylitis mouse model of Bardos T. et al was modified for BALB/c mice and C57BL/6J (C57) mice (Charles River). Human proteoglycan was subcutaneously injected in complete Freund's adjuvant at week 0 and in incomplete Freund's adjuvant at week 2 and 4. At week 34, magnetic resonance imaging (MR) of the sacroiliac joints was performed (3T Magnetom Skyra, Siemens), levels of cytokines were determined in heparin-plasma by Luminex ProcartaPlex Mouse Panel 1 (eBioscience), and Wnt3a and DKK1 were quantified by ELISA kits (Abbexa Ltd. and R&D Systems). Statistical analysis was performed with SPSS (version 22.0; SPSS, Inc.). Data are shown as mean±SD or median/range depending on the distribution of the values.
Results: Immunized C57 mice (n=12) had higher levels of DKK1 compared to their controls (n=7) (7417 ± 4783 vs. 2303 ± 893, p=0,004), and immunized BALB/c mice (n=7) tended to have lower levels of wnt3a compared to immunized C57 mice (2626/762-20000 vs. 9897/3055-50327, p=0,020); levels within strains were comparable. Wnt3a negatively correlated with DKK1 in immunized BALB/c mice (Rho: -0,929, p=0,003) but not in control BALB/c (n=6) or C57 mice. Immunized C57 mice more frequently developed bone edema than immunized BALB/c mice (p=0,017) and their controls (p=0,031). Subgrouping in MR+ and MR- mice revealed that immunized MR+ BALB/c mice tended to have higher wnt3a and lower DKK1 levels compared to MR- mice and immunized MR+ C57 mice (12485/4970-20000 vs. 1950/763-2627, p=0,064; 2039/1959-2119 vs. 8516/5028-15004, p=0,064 and 7864/736-15356, p=0,076, respectively). On the cytokine level, IL-17A positively correlated with DKK1 in immunized BALB/c and C57 mice (Rho: 0,757 p=0,049; Rho: 0,847 p=0,001), but not with TNF-α. IL-17A showed a strong trend for a negative correlation with wnt3a (Rho: -0,739, p=0,058) in immunized BALB/c mice but not in C57 mice.
Conclusion: These results suggest differences in the expression patterns of inflammatory cytokines and osteogenic factors in BALB/c and C57 axSpA models, with different results depending on sacroiliac edema. DKK1 positively correlates with IL-17A but not with TNF-α in both immunized mice strains, indicating a major role of IL-17A in the late stages of murine axSpA models.

Orphan receptor NR2F6 in germinal center reaction - implication for autoimmunity

William Olson, Victoria Klepsch, Verena Labi, Gottfried Baier, Natascha Hermann-Kleiter

Objective: Follicular CD4+ helper T (Tfh) cells provide classical help for B cells in order to facilitate production of high affinity antigen-specific memory B and plasma cells within germinal centers (GC). During their differentiation from naïve CD4+ T cells, Tfh cells face the challenge of balancing stability and plasticity in their gene expression programs while under the influence of their microenvironment. The transcription factors that regulate the differentiation of Tfh cells remain incompletely understood. Tight control of T follicular helper cells is required for optimal maturation of the germinal center response during infection. However, over-reactivity can lead to production of autoantibodies and autoimmune disease.
Results: We have previously established the nuclear orphan receptor NR2F6 as a safeguard against Th17 derived autoimmunity (Hermann-Kleiter et al., 2008 and 2012) on one side but as a check on Th1 CD4+ and CD8+ T cells activity during cancer immune surveillance on the other side (Hermann-Kleiter & Klepsch et. al., 2015). In humans Nr2f6 expression in CD19+ B cells derived from systemic lupus erythematosus patients is significantly reduced (Hutcheson J, et al., 2008). In order to investigate if NR2F6 plays a more general role in the regulation of autoimmunity we want to investigate the two key players of the humoral adaptive immune system e.g. T follicular and B cells. In vitro NR2F6 suppresses the cytokines produced by Tfh cells interleukin (IL)-4 and IL-21. Loss of Nr2f6 results in enhanced numbers of Tfh cells and germinal center B cells within the spleen after sheep red blood cell (SRBC) immunization.
Outlook: Define the cell intrinsic role of NR2F6 in CD4+ Tfh and B cells ex vivo and in vivo analyzing wild type and Nr2f6-deficient as well as OT-II-transgenic mice.

william.olson@i-med.ac.at

The *Aspergillus fumigatus* leucine biosynthesis-regulator LeuB is crucial for adaptation to iron starvation and virulence in *Galleria mellonella*

The mould *Aspergillus fumigatus* is the most common airborn fungal pathogen of humans causing allergic reactions and severe invasive diseases in immunocompromised patients. In order to identify potential novel targets for antifungal therapy, we investigated the mechanisms involved in biosynthesis and regulation of the amino acid leucine, which represents an essential amino acid for humans. Therefore, we generated three *A. fumigatus* mutant strains lacking either the leucine biosynthetic enzymes LeuA (α-isopropylmalate isomerase, Afu2g11260) or LeuC (isopropylmalate synthase, Afu1g15000), or the leucine regulatory transcription factor LeuB (Afu2g03460). Deficiency in either LeuA (strain ΔleuA) or LeuC (strain ΔleuC) resulted in leucine auxotrophy, whereby the ΔleuC mutant required significantly higher leucine supplementation for growth than the ΔleuA mutant. Deficiency in LeuB (strain ΔleuB) resulted in partial leucine auxotrophy, i.e. the mutant was able to grow without leucine supplementation but required leucine supplementation for full growth. Interestingly, the ΔleuB mutant displayed significantly decreased resistance to iron starvation. In the *Galleria mellonella* infection model, deficiency of LeuA, LeuB and particularly LeuC attenuated virulence of *A. fumigatus*. In conclusion, these data demonstrate that leucine metabolism is a virulence determinant of *A. fumigatus* and reveal an unprecedented crosstalk between leucine and iron metabolism.

Thomas Orasch[1], Sophie Pleifer[1], Ulrike Binder[2], Fabio Gsaller[3], Michael Bromley[3], Hubertus Haas[1]

[1]Department of Molecular Biology, Medical University of Innsbruck, Austria
[2]Division of Hygiene and Medical Microbiology, Medical University of Innsbruck, Austria
[3]The University of Manchester, Manchester Academic Health Science Centre, Manchester, UK. The Manchester Fungal Infection Group, The University of Manchester, Manchester, UK

thomas.orasch@i-med.ac.at

Poster # 45 immunology

Cbl-b inhibition serves as sensitizer effect in the established CTLA-4 checkpoint blockade cancer immunotherapy

Sebastian Peer, Thomas Gruber, Gottfried Baier

Medical University of Innsbruck, Department of pharmacology and genetics

sebastian.peer@student.i-med.ac.at

Modulation of the immune system for the treatment of primary and metastatic tumours in cancer patients has been a goal for many decades. The E3 ubiquitin ligase Cbl-b has been established to mediate both the thresholds of the CD28 and the TGFβ receptor signaling pathways during T cell activation, critically contributing to the maintenance of self-tolerance.
Based on own published and unpublished findings, that Cblb-deficient mice are able to immunologically reject otherwise lethal tumor burdens, we have identified and preclinically validated the Cbl-b as a bona fide immune checkpoint in vivo. Mechanistically, we now demonstrate drastically elevated GM-CSF production of Cblb-deficient CD4+ T-cells when compared to wild-type control cells. This is of high interest, as preclinical studies have suggested that immune cell produced GM-CSF induces host-protective cancer immunity.
Because very recently, blockade of i.e. immune checkpoints CTLA-4 and PD-1 has emerged as promising strategy in the development of effective cancer immune therapies, we explored the concept of targeting different checkpoints blockade therapy regimens concomitantly. As result, we observe that CTLA-4- (but not PD-1-) based immunotherapies selectively benefit from modulation of the immunosuppressive Cbl-b pathway in vivo. This concept to synergistically improve immune functions appears of high clinical impact in order to further develop improved immune-oncological regimens.

Human dendritic cells induce divergent immune responses according to the allergenic potential of two homologous lipocalins: Identification of mechanisms shaping dendritic cells to induce an allergic Th2 immune response

immunology Poster # 46

Beate Posch[1], C. Irsara[1], M. Herrmann[2], D. Fuchs[3], D. Bindreither[4], N. Reider[1], B. Redl[5], M. Nussenzweig[6], L. Von-Boehmer[6], C. Heufler[1]

[1]Department of Dermatology
[2]Department of Anaesthesiology and Critical Care Medicine
[3]Division of Medical Biochemistry, Biocenter,
[4]Division of Molecular Pathophysiology, Biocenter
[5]Division of Molecular Biology, Biocenter, Medical University Innsbruck, Austria
[6]Rockefeller Universirty, USA

beate.posch@i-med.ac.at

Background: Why and when the immune system initiates TH2 mediated allergic responses is still insufficiently characterized. One of the key players during the induction of allergic airway inflammation providing stimuli for TH2 cell differentiation are dendritic cells (DCs). We investigated the interaction of human monocyte derived DCs with lipocalins, a protein family comprising the majority of mammal derived respiratory allergens. We employed allergens possessing a high sequence homology with endogenous human lipocalins. Specifically we worked with the dog allergen Can f 1 and its human non-allergenic homologue Lipocalin-1 (Lcn-1) and the cat allergen Fel d 4 and its putative human homologue the major urinary protein (MUP).
Methods: Factors involved in directing the type of immune responses including antigen uptake, maturation induction, tryptophan breakdown and cytokine production by human monocyte derived dendritic cells were determined. The type of induced immune response was characterized in DC - T cell co-cultures measuring key cytokine secretion of T cells. Additionally, a microarray of dendritic cells treated with the lipocalins (Lcn-1 or Can f 1) and a subsequent global gene expression reactome pathway analyses were performed. Allergen specific antibodies were produced from Can f 1 or Fel d 4 allergic donors via the single B-cell cloning approach.
Results: The two homologous lipocalins had differential effects on DCs according to their allergenic potential. The allergens Can f 1 and Fel d 4 persistently induced less of the Th1 skewing maturation marker expression, tryptophan breakdown and IL-12 production in human monocyte derived DCs when compared to the endogenous non-allergenic Lcn-1 or MUP. As a consequence, T cells stimulated by DCs treated with Can f 1 or Fel d 4 produced more of the Th2 signature cytokine IL-13 and less of the Th1 signature cytokine IFN-γ than T cells stimulated by Lcn-1 or MUP treated DCs. Microarray data supported the functional analyses (IL12, IDO, some maturation marker genes). Reactome pathway analyses of the gene expression data revealed differences in the intracellular trafficking, sorting and antigen presentation pathways of allergen or non-allergen treated DCs. Those data led us to hypothesize that the endosomal system plays a decisive role for the induction of a specific immune response upon lipocalin challenge. To track the processing pathway of the allergens we produced allergen specific antibodies form allergic donors via the single B-cell cloning approach. The endosomal processing pathways will be further analysed via various tracking experiments with markers for phagosomal/endosomal maturation and acidification.
Conclusion: Our data contribute to the field by showing that human monocyte derived dendritic cells orchestrate immune responses in responding differentially to four highly homologous lipocalins according to their allergenic potential. The crosstalk of dendritic cells with lipocalins alone has the potential to direct the type of induced immune response and the endosomal processing system seems to play a crucial role during this decision process.

Intradermal injection of human antibodies against lectin receptors targets defined subsets of dendritic cells in human skin

Sandra Schaffenrath[1,5], Barbara del Frari[2], Kerstin Komenda[1], Gabriel Djedovic[2], Christian Münz[3], Tibor Keler[3], Patrizia Stoitzner[1], Nikolaus Romani[1,5]

[1]Department of Dermatology & Venereology
[2]Department of Plastic, Reconstructive and Aesthetic Surgery, Medical University of Innsbruck, Austria
[3]Viral Immunobiology, Institute of Experimental Immunology, University of Zürich, Switzerland
[4]Celldex Therapeutics Inc., Needham, MA, USA
[5]Oncotyrol – Center for Personalized Cancer Medicine, Innsbruck, Austria

sandra.schaffenrath@i-med.ac.at

Dendritic cells (DC) are essential for the induction of primary immune responses, and hence preferred targets for cancer immunotherapy. Skin DC express C-type lectin receptors for recognition and binding of pathogen-derived antigens. Langerhans cells (LC) express mainly Langerin/CD207, whereas DEC-205/CD205 is expressed by dermal DC and at low levels also on LC. CD14+ dermal monocyte-derived DC posses the mannose receptor (MR/CD206). We used an ex vivo approach for targeting DC in situ with monoclonal antibodies (mAb) against these receptors. The targeting mAbs were injected intradermally into human skin explants. Migratory skin DC carried targeting mAb from skin explants into the culture medium over a period of 3-4 days. Corresponding to the expression patterns of these lectin receptors in skin DC, anti-Langerin mAb was detected exclusively in epidermal LC, DEC-205 mainly in CD1a+/CD14- dermal DC and MR in both subsets of dermal DC, somewhat more in the CD14+ population. Anti-Langerin and anti-MR mAb persisted intracellularly in migratory DC for up to 4 days. Human anti-human and mouse anti-human DEC-205 mAb showed identical targeting patterns. Since effective vaccination requires the addition of adjuvant we co-administered the TLR-3 ligand poly I:C. This enhanced uptake of DEC-205 mAb by the targeted skin DC subsets, whereas Langerin targeting was unchanged, and MR targeting even decreased due to some maturation. Our findings demonstrate that LC can be preferentially targeted by Langerin mAb, in contrast DEC-205 mAb can also be bound by dermal skin DC subsets to varying degrees. The efficacy of DEC-205 targeting can be boosted by poly I:C. Cancer vaccines consisting of tumor-antigen / anti-DC antibody conjugates will extend patients' pre-existing immunity by eliciting tumor-specific T cells de novo. They may ultimately reveal their full therapeutic potential with the help of modern immune checkpoint inhibitors.

Ex vivo reversal of the effects of Rivaroxaban evaluated using ROTEM thromboelastometry and thrombin generation assay

Bettina Schenk[1], P. Würtinger[2], W. Streif[3], W. Sturm[4], D. Fries[1], M. Bachler[1]

[1]Medical University of Innsbruck, Department of General and Surgical Intensive Care Medicine, Innsbruck, Austria
[2]Medical University of Innsbruck, Central Institute for Medical and Chemical Laboratory Diagnostics, Austria
[3]Medical University of Innsbruck, Department of Pediatrics, Innsbruck, Austria
[4]Medical University of Innsbruck, Department of Internal Medicine, Innsbruck, Austria

bettina.schenk@i-med.ac.at

Background: In major bleeding events, the new direct oral anticoagulants (DOACs) pose a great challenge for physicians. Aim of the study was to test for the effective ex vivo reversal of the DOAC rivaroxaban with various non-specific reversing agents: PCC (prothrombin complex concentrate), aPCC (activated prothrombin complex concentrate), rFVIIa (recombinant activated factor seven) and FI (human fibrinogen concentrate).
Patients/Methods: Blood was drawn from healthy volunteers and from patients treated with rivaroxaban. Blood samples from healthy volunteers were spiked with rivaroxaban to test the correlation between rivaroxaban level and coagulation tests. Patient blood samples were spiked with various concentrations of the above-mentioned agents and analysed using thromboelastography and thrombin generation.
Results: When added in vitro, rivaroxaban significantly correlated with thromboelastometry EXTEM (extrinsic coagulation pathway) clotting time (CT) and time to maximum velocity (MaxV-t) and with all measured thrombin generation (TG) parameters ($p<0.001$ for all correlations). In vivo, ROTEM CT and MaxV-T, and TG lag time and peak thrombin generation (Cmax), were significantly correlated with rivaroxaban levels. Regarding reversal of rivaroxaban, all tested agents significantly reduced EXTEM CT, but to different extents: rFVIIa by 68% and aPCC by 47%, PCC by 17% and FI by 9%. Only rFVIIa reversed EXTEM CT to baseline values. Regarding TG, PCC (+102%) and aPCC (+232%) altered overall thrombin generation (AUC) and increased Cmax (+461% for PCC and +87.5% for aPCC). Lag time was not reversed by any of the tested agents, but further prolonged ($p<0.05$) by FI (+27%) and by PCC (+33%). Time to peak thrombin activity was also further prolonged by FI (+26%) and by aPCC (+96%).
Conclusions: Our data indicate that great care must be taken when analysing data gained in vitro and translating such data to in vivo situations. After all, it is not clear which reversal agent is best for the treatment of bleeding patients under rivaroxaban since TGA and ROTEM do not favour the same agents. EXTEM CT is a potentially useful parameter to detect rivaroxaban and its (non-specific) reversal. TG was less conclusive in this study but this may depend on the assay and reagents used.

LCMV-GP Pseudotyped Oncolytic Vesicular Stomatitis Virus for Lung Cancer Therapy

Lisa-Marie Schreiber[1], I. Winkler[1], P. Erlmann[1], C.R. Urbiola[1], S. Köck[2], J. Kern[2], H. Zwierzina[2], D. Holm-von Laer[1], G. Wollmann[1]

[1]Division of Virology, Department of Hygiene, Microbiology and Social Medicine, Innsbruck Medical University, Innsbruck, Austria
[2]Tyrolean Cancer Research Institute, Translational Cancer Research, Innsbruck, Austria

Lung cancer is the leading cause of cancer deaths worldwide and claims more lives each year than do colon, prostate, ovarian and breast cancers combined. Standard treatment options include surgery, radiation and chemotherapy, however, impact on survival in advanced stages is limited. A new innovative treatment approach is the use of oncolytic viruses that act through selective targeting and killing of tumor cells and through stimulation of an anti-tumor immune response. Among currently studied oncolytic viruses, vesicular stomatitis virus (VSV) is a particularly candidate due to its fast mode of action, high titer production, absence of pre-existing anti-VSV immunity and broad tumor tropism. However, potential neurotoxicity of wild-type VSV and a rapid induction of neutralizing antibodies have been a hindrance for further clinical advancement. Our group previously reported that pseudotyping VSV with the LCMV glycoprotein (VSV-GP) results in complete abrogation of neurotoxicity creating a safe and highly efficient oncolytic virus. Furthermore, the absence of neutralizing antibody induction by VSV-GP paves the path for repetitive therapeutic application.
Here, we propose the use of oncolytic VSV-GP for the treatment of early-stage lung cancer.
In a first set of experiments, the oncolytic activity of VSV-GP was assessed in vitro on a variety of lung cancer cell-lines and it was found to efficiently infect and lyse most of the cell-lines. However, analysis of the innate immune response of lung cancer cells to VSV-GP revealed IFN type I production and induction of an antiviral state of the cells as a potential mechanism leading to shortcomings in virotherapeutic treatment. Weakening the antiviral response by knocking out Interferon Receptor 1 (IFNAR1) on mouse lung cancer cells, VSV- GP showed significantly stronger oncolytic activity in vitro, but was able to only minimally slow down tumour growth in a LLC-1 IFNAR1-/- subcutaneous syngeneic model in vivo. Therefore further studies will be necessary to better understand how the oncolytic effect, the IFN-I and anti-tumour immune response interact and what strategies will result in enhanced therapeutic outcome to optimize the efficiency of oncolytic VSV-GP in lung cancer. Additional studies are underway using in vitro spheroid cultures and in vivo models to assess the impact of the tumor - tumor stroma interaction on the virus' ability to infect, kill and spread through the tumor.

High yield expression system for the antifungal protein PAF

Christoph Sonderegger[1], Ádám Fizil[2], Gyula Batta[2], Florentine Marx[1]

[1]Medical University of Innsbruck, Dept. of Molecular Biology, Innsbruck, Austria
[2]University of Debrecen, Dept. of Organic Chemistry, Debrecen, Hungary

christoph.sonderegger@i-med.ac.at

Considering the steadily rising number of immuno-compromised patients and the high risk for the development of drug resistance, antimicrobial proteins are gaining increased attention as promising new therapeutics to prevent and/or treat microbial infections. Especially novel drugs against fungal pathogens are urgently needed, since the mortality rate (~1.5 million per year) is even outnumbering the deaths caused by tuberculosis or malaria. A promising candidate for the development of novel pharmaceuticals is the small, cationic and cysteine-rich antifungal protein PAF from the β-lactam producer Penicillium chrysogenum. For a possible future medical application it is indispensable to investigate the mechanism of action and the structure-function relationship of PAF in more detail to potentially enhance its toxicity by protein modelling. NMR technology for the investigation of the structure requires milligrams of isotopically labelled and purified protein of high quality because impurities disturb structural analyses. Our study focused on the production of antifungal protein variants applying a Penicillium chrysogenum-based expression system that ensures sufficient amount and optimal purity for NMR technology.
To study the role of distinct protein motives we generated several PAF protein variants in which specific amino acids – predicted by in silico analyses to have crucial functions – were exchanged by PCR-based site-directed mutagenesis. The mutated paf-genes were cloned into the vector pSK275λpaf containing the strong paf-promotor. P. chrysogenum itself is resistant against PAF. Therefore, a P. chrysogenum paf-deletion strain served as recipient for plasmid transformation. Protein production was conducted in liquid culture for three days and secreted PAF variants were purified from supernatants via cation-exchange chromatography. By using this efficient expression system protein yields of 80 mg/l and above could be reached. The obtained PAF protein variants were characterised for their solution structure using NMR and the antifungal activity was determined in comparison to the native PAF.
The P. chrysogenum mutant Δpaf served as perfect microbial expression factory and the use of defined minimal medium allowed an easy one-step purification of the recombinant proteins. Mass spectrometry and NMR proved the high quality, correct processing and folding of the produced proteins. By investigating their antifungal activities we found that some of the amino acid exchanges led to a significant loss of activity, while other mutations had obviously no effect. This study provides new insights into protein motives that are important for the antifungal function of PAF and forms the basis for further protein modifications for activity enhancement.

Laser-assisted topical immunization with antigen-antibody complexes to target skin dendritic cells

Christoph H. Tripp[1], Hermann Voit[1], Michael Lohmüller[1], Juliana Idoyaga[2], Patrizia Stoitzner[1]

[1]Department of Dermatology, Venereology and Allergology, Medical University of Innsbruck, Innsbruck, Austria
[2]Microbiology and Immunology Department, Stanford University School of Medicine, Stanford, CA, USA

christoph.tripp@i-med.ac.at

Skin dendritic cells (DC) are very potent antigen presenting cells and the prime cells to induce immune responses against cutaneous infection and tumours. Antigen can be targeted to DC with the help of antibodies against surface molecules, such as the lectin receptors DEC-205 and Langerin. We know from preliminary results that antibody-antigen complexes penetrate poorly into barrier-disrupted skin as achieved by repeated tape stripping of skin. For improved delivery we tested laser poration with the infrared laser device from Pantec Biosolutions AG (P.L.E.A.S.E.®) that generates aqueous micro-pores of defined depth in the skin. Through these newly formed pores it should be possible to deliver larger molecules such as antibody-antigen conjugates for immunization. We immunized mice with DEC-205-OVA or Langerin-OVA through laser-treated ear skin in comparison to intradermal application and measured activation of antigen-specific CD8+T cells a week later. After intradermal immunization with DEC-205-OVA we detected higher numbers of pentamer+ CD8+ T cells than with a control antibody conjugated to OVA. This correlated with enhanced cytotoxic T cell responses in vivo. Langerin-OVA immunization was less effective as we have shown earlier. When we checked the penetration and transport of fluorescence-conjugated anti-Langerin and anti-DEC-205 antibodies after laser pretreatment of skin we observed that antigen-antibody complexes are not taken up by skin DC. We are currently optimizing the settings for laser poration to investigate the full potential of this immunization approach.

Effects of iron imbalances on mitochondrial activity in vivo

Chiara Volani[1], D. Haschka[1], E. Demetz[1], C. Doerrier[2], E. Gnaiger[2], G. Weiss[1]

[1]Dept. of Internal Medicine VI, Medical University of Innsbruck, Austria
[2]Oroboros Instruments, Innsbruck, Austria

chiara.volani@i-med.ac.at

Background: Mitochondria are dynamic organelles, involved in fundamental cell processes, including oxidative phosphorylation. Iron plays a decisive role in these processes because it is central part of mitochondrial enzyme complexes but also regulates citric acid cycle activity by modulating mitochondrial aconitase expression. Hence, imbalances of iron homeostasis impact on mitochondrial activity and, thus, on cell and organ functions.
So far, little information is available on how to best measure tissue mitochondrial activity and its interaction with iron homeostasis in vivo; therefore we questioned whether the assessment of mitochondrial respiration in peripheral blood mononuclear cells (PBMCs) could be a good surrogate marker for that.
Methods: Human PBMCs were collected from buffy coats, purified cells (2x10^6 cells/ml) were resuspended in mitochondrial respiration medium (MiR05), and mitochondrial activity was performed by high resolution respirometry (OROBOROS Instruments, Austria). Moreover, to investigate the impact of iron on mitochondrial respiration we studied mitochondrial function in livers of mice, receiving either iron deficient- or standard iron-diet two or four weeks before being sacrificed. The liver was collected and stored in Custadiol prior to homogenization in MiR05. Mitochondrial leak respiration, complex I and II maximal oxidative phosphorylation together with non-coupled respiration of the homogenates were assessed at a final concentration of 2 mg.
Results: Our ongoing experiments indicate that mitochondrial function testing can be successfully performed in human PBMCs as well as in mouse tissues. Analyses of liver samples from mice indicate that dietary iron supplementation triggers changes in oxidative phosphorylation, and has a direct impact on the activity of the electron transfer system complexes.
Conclusion: The use of high-resolution respirometry (OROBOROS Instruments, Austria) represents a powerful and reliable tool to investigate mitochondrial respiration in PBMCs, which might provide useful information on the tissue mitochondrial activity.

Satb2 Links Synaptic Activity Dependent Changes in Nuclear Geometry with Long-Term Memory Formation

Andreas Abentung, I. Cera, C. Jaitner, C. Reddy, P. Feurle, G. Apostolova, G. Dechant

Institute for Neuroscience, Medical University Innsbruck

andreas.abentung@i-med.ac.at

In hippocampal neuronal cultures synaptic activity stimulates the formation of nuclear infoldings through an unknown mechanism that is triggered by calcium entry (Wittman et al., 2009). We found that the nuclear DNA binding protein "Special AT-rich sequence binding protein 2" (Satb2) interacts with proteins of the inner nuclear membrane, which are known regulators of nuclear shape. Therefore we have investigated the role of Satb2 in activity-dependent plasticity of nuclear morphology of hippocampal neurons.
We found that Satb2 is regulated by neuronal activity as well as BDNF in primary hippocampal cultures. The genetic deletion of Satb2 prevents changes of nuclear morphology observed during increased synaptic transmission or upon addition of BDNF in hippocampal cultures. Overexpression of Satb2 in vitro increases the number of nuclear infoldings in unstimulated cultures.
In the adult CNS Satb2 is specifically expressed in pyramidal neurons of the CA1 field of the hippocampus and the neocortex. Satb2CamKIICre conditional mouse mutants, in which Satb2 is selectively deleted in the adult forebrain, have deficits in L-LTP and long term memory formation. In addition, the number of nuclear infoldings is greatly reduced in CA1 of Satb2CamKIICre conditional knock-out mice. Nuclear dysmorphology is rescued together with long-term memory deficit when Satb2 is re-expressed in the hippocampus of Satb2 conditional mutants.
Based on our results we hypothesize that activity-dependent regulation of Satb2 protein levels in the nucleus of hippocampal neurons modulates association of specific chromatin loops with the inner nuclear membrane and consequently regulates genes expression underlying the consolidation of memory.

Neuropeptides Treatment Options in Temporal Lobe Epilepsy (TLE)

Alexandra Sylvia Agostinho[1], Mario Mietzsch[2], Luca Zangrandi[1], Regine Heilbronn[2], Christoph Schwarzer[1]

[1]Department of Pharmacology – Medical University of Innsbruck
[2]Institute of Virology – Charité – Medical University of Berlin

alexandra.agostinho@i-med.ac.at

The high incidence of drug-resistant focal epilepsies poses a persistent challenge in medicine. Certain patients benefit from surgical removal of the epileptogenic focus. However, a large cohort of patients cannot be successfully treated. The importance of endogenous peptides in seizure control is widely acknowledged, however long-term treatment is needed in epilepsy. To achieve this, viral vector derived, locally restricted continuous expression of neuropeptides was evaluated as treatment option for focal epilepsy in a pharmaco-refractory model of TLE.
We analyzed the effects of specific peptides expressed in the epileptogenic focus after unilateral injection of kainic acid into the dorsal hippocampus of mice. The onset, frequency and duration of seizure related events like sharp waves, bursts and paroxysmal discharges were measured by in-vivo EEG recordings. Behavioral tests focusing on spatial memory abilities and emotional control were performed to investigate brain functions known to be impaired both, in patients suffering from the disease and in animal models of TLE.
Neuropeptide expression led to suppression of generalized seizures and hippocampal paroxysmal discharges up to 6 months after injection (currently the longest time interval investigated). Moreover, treatment of mice 1 or 2 weeks after kainic acid injection conserved spatial memory ability (Barnes maze) up to 6 months, while control animals lost this ability already after 1 or 2 months.
The long-term goal of our studies is to develop the preclinical model into a gene therapy protocol for patients suffering from refractory mesial temporal lobe epilepsy, and potentially other types of intractable, focal epilepsies.

A role for Cav1.4 L-type calcium channels in the pineal gland

Kathleen Amberg[1], Dagmar Knoflach[2], Hartwig Seitter[1], Alexandra Koschak[1]

[1]University of Innsbruck, Institute of Pharmacy, Pharmacology and Toxicology, Innsbruck
[2]Medical University Vienna; Department of Neurophysiology and Pharmacology, Vienna

kathleen.amberg@uibk.ac.at

The pineal gland, also known as the third eye, is involved in regulating circadian rhythm by secreting melatonin (N-acetyl-5-methoxy-tryptamine), a neuroendocrine hormone, which plays a pivotal role to regulate the sleep wake cycle. At night noradrenergic input reached the pinealocytes and regulates the production of the messenger melatonin, which is released into the blood stream. Voltage-gated calcium channels participate in the physiological signalling of pinealocytes and may be involved in regulation of the secretion night-hormone melatonin. Among the group of L-type calcium channels (LTCCs) we show that Cav1.4 were the predominant channels in pinealocytes.
The overall aim of the study is to determine the influence of Cav1.4 LTCCs on melatonin production. We are therefore establishing conditions for the cultivation of pineal glands and ELISA measurement of melatonin. For the measurement of melatonin we were using the pineal gland tissue, cultivated in cell-medium stimulated with or without norepinephrine for 24hours. The melatonin concentration of different samples (plasma, glands, culture medium) from wild type and Cav1.4 mutant mice (IT, KO) was determined by a competitive ELISA. We report here that the plasma levels of melatonin were found to be in range of published data. The culture supernatant contained very high levels of melatonin. All genotypes yielded high amounts of melatonin in culture but it is still unclear if there are differences between genotypes (IT vs KO vs WT). So far the variability of the ELISA is relatively high and it might not be precise enough to see small differences.
The successful tissue cultivation and melatonin measurement secreted from mouse pineal glands is an important methodological prerequisite to determine the role of Cav1.4 LTCCs in the pineal gland. In our further experiments we will also determine Cav1.4 expression and localisation in pinealocytes.

The role of Nogo66 receptor family members (NgRs)during development of PNS circuits

Bajic Danica, Bäumer Bastian, Herzog Chiara, Bandtlow Christine

Division of Neurobiochemistry, Medical University Innsbruck, Austria

danica.bajic@i-med.ac.at

The somata of various somatosensory neurons—including mechanoreceptors, thermoreceptors, and nociceptors—are intermingled in the dorsal root ganglion (DRG). During development, axons of sensory neurons of the DRG project to both a specific peripheral target such as skin or skeletal muscle, and to different second-order neurons within the spinal cord. As axons extend into the periphery they encounter a plethora of target-derived signals that they must interpret properly in order to accomplish axonal guidance, branching, maturation, target selection, synapse formation and survival of morphologically and functionally distinct subset of sensory neurons. Although it is well established that growth factors and their receptors regulate – at least in part - these processes, the molecular mechanisms that govern the specificity of axonal growth, guidance and selective target innervation of distinct populations of somatosensory neurons remains unclear. Previously we could show that NgR2, a member of the Nogo66 family of guidance receptors (NgR) and abundantly expressed by sensory neurons, is especially important for shaping peripheral skin innervation by nonpeptidergic nociceptors. NgR2 mediates axon-repulsive activities of the extracellular matrix molecule Versican, which is expressed at the dermal-epidermal border (Bäumer et al., 2014) and specifically prevents epidermal innervation of a subclass of nonpeptidergic neurons. The developmental and functional nociceptive defects associated with loss of NgR2 manifest behaviorally in increased sensitivity to noxious mechanical pressure and cool temperatures.
Here, we show that NgR1;NgR2 double null mice display a significant deficit in survival of myelinated sensory neurons at P1 and P15. Specifically, medium-sized trkB-positive neurons are affected. TrkB-expressing neurons are A -low-threshold mechanoreceptors (LTMRs) that innervate the most abundant skin hair follicles and are crucial for touch sensation. Indeed, when studying hair follicles in mouse back skin, we observed that in NgR1;NgR2 double null mice the quantity of lanceolate endings innervated by axons coexpressing NF200+/trkB+ was significantly reduced. Whether other mechanoreceptor end organs, such as Merkel cell-neurite complexes and Meissner corpuscles are similarly affected is presently under investigation. Moreover, to understand the mechanisms underlying the innervation deficits in NgR1;NgR2 double null mice, we will analyse whether trk signals are modulated by NgRs to confer cell type-specific responses to their respective ligands during development.

Behavioral characterization of the anterior injection model of subarachnoid hemorrhage

Susanne Boettinger, F. Kolk, G. Broessner, R. Helbok, B. Pfausler, E. Schmutzhard, R. Beer, P. Lackner

Background: The anterior injection model is an accepted murine model for the pathophysiology of subarachnoid hemorrhage (SAH). So far it is not well characterized regarding the applicability of neurological scores. Therefore this study was performed to evaluate three different behavioral tests for quantifying disease severity.
Methods: Forty-seven C57BL/6 (10-12 weeks old) mice were stereotaxically injected with different amounts of autologous arterial blood into the prechiasmatic cistern. SHAM control mice underwent the same procedure without blood injection. The following two or seven days after surgery respectively, mice underwent different behavioral test (Flex-field and beam-balance analysis, SHIRPA score). Subsequently brains were analyzed by histology (TUNEL, Fluoro-Jade-C, Fibrinogen, GFAP).
Results: Flex-field analysis of SAH animals showed a significant reduction of light barrier breaks compared to controls in the first two days after SAH. This reduction was more intense in animals with a higher amount of injected blood. The SHIRPA score revealed a significant reduction in muscle tone and body temperature in SAH animals two days after surgery. A significant increase of GFAP expression was observed in SAH animals compared to SHAM controls in the early phase of SAH. Furthermore Fibrinogen staining indicated a significantly increased number of microthrombi in SAH animals at this time point. Moreover there was a significantly increased number of Fluoro-Jade-C and TUNEL positive neurons in SAH mice suggesting neurodegeneration in the early phase of SAH. In contrast, increased staining of all markers was observed but failed to reach significance 7 days after SAH.
Conclusion: The results of flex field analysis and SHIRPA score show behavioral and physiological deficits in the first two days after SAH in parallel to histological alterations indicating neuronal damage. In summary these tests can be used as functional outcome parameters in the anterior injection model of SAH.

susanne.boettinger@i-med.ac.at

Meningeal-derived oligodendrocytes transplantation improves clinical outcome in spinal cord injury rat model

In most diseases of the spinal cord, demyelination plays an important role in both generation and progression of the neurodegenerative lesion; in addition, endogenous oligodendrocytes regeneration is apparently insufficient for repair. Transplantation of oligodendrocytes represents a promising avenue for treatment of demyelinating disorders, although clinical application is hindered by the lack of adequate cell sources. Our group has described a population of neural stem/progenitor cells that resides in the adult leptomeninges and can be isolated and induced to differentiate into neural and glia lineages. In this work we aim to obtain a large number of transplantable oligodendrocytes from small meningeal biopsies and to test their regenerative potential in a contusive spinal cord injury rat model. We expanded meningeal neural stem cells from organotypic culture of small (1-3 mm2) meningeal biopsies. By developing a highly efficient multi-step protocol, we differentiated meningeal-derived neurospheres into oligodendrocytes. Meningeal-derived oligodendrocytes acquired the typical oligodendroglial morphology and expressed the myelin-specific markers at protein and gene expression levels.
To assess the regenerative potential of meningeal-derived oligodendrocytes, we transplanted 6x105 eGFP-labelled cells in a contusive model of spinal cord lesion 6 days after the injury. Evaluation of functional recovery with the Basso, Beattie and Bresnahan (BBB) rating scale and subscale and Catwalk gait analysis evidenced a statistically significant enhancement in locomotor recovery in animals injected with meningeal-derived oligodendrocytes. In order to establish the mechanisms through which transplanted cells might have contributed to a better functional recovery, we analysed the localization and phenotype of eGFP+ cells in spinal cord samples. Histological analysis revealed that 1 day post transplantation, eGFP meningeal-derived oligodendrocytes were localized at the injury site, however 2 months later they migrated rostrally and caudally and accumulated in meninges. We didn't observe any differences in cyst volume between meningeal-derived oligodendroctyes transplanted and control injured rats, suggesting that their beneficial effect may rely on neuroprotective properties.
In conclusion, we have established an efficient and reproducible method to generate large numbers of oligodendrocytes from a small meningeal biopsy; in addition, the evidence of a correlation between meningeal-derived oligodendrocytes transplantation and improvement of functional recovery in a rat model of contusive spinal cord injury, suggests that meningeal-derived oligodendrocytes may play a role in regenerative therapies of demyelinating disorders.

Sissi Dolci, V. Berton, A. Braga, F.J. Rodríguez, P.H. González-Sánchez, G. Malpeli, A. Pino, Krampera M., F. Bifari, G. Fumagalli, I. Decimo

sissi.dolci@gmail.com

Modeling human peripheral sensory neurogenesis and Friedreich ataxia with pluripotent stem cells

Andreas Eigentler[1], Anita Erharter[1], Zoe Puschban[1], Sylvia Boesch[2], Carlo Bavassano[1], Georg Dechant[1], Roxana Nat[1]

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

andreas.eigentler@i-med.ac.at

Background: The potential of pluripotent stem cells (PSCs) to differentiate into any cell type of the body has been widely exploited. Particularly, patient-derived induced pluripotent stem cells (iPSCs) have been proven useful for modeling neurological diseases in vitro.
However, the accurate recapitulation of human neurogenesis in vitro remains a challenge and requires a precise understanding of the development of each neuronal subtype by means of accurate criteria to characterize the generated cells. We aim to model human peripheral sensory neurogenesis by following the differentiation of PSCs and the developmental principles demonstrated in animal models. This in vitro system will be used to reveal pathophysiological processes in Friedreich ataxia (FRDA), a hereditary neurodegenerative disease affecting primarily dorsal root ganglia neurons.
Methods: We applied a protocol for directed differentiation of human PSCs (ESC and iPSCs from FRDA patients and healthy controls) into neural crest (NC) cells and peripheral sensory neurons (PSNs) (Eigentler et al. 2014; Stem Cells Dev 22(24):3271-82). We provided evidence for NC and PSN identity via qPCR and immunocytochemistry for markers of dorsal root ganglia in human embryos (Betters et al. 2010; Dev Bio 344(2):578-92): TFAP2, P75NTR, SOX9, SOX10, NGN1, NGN2, PRPH, BRN3A, ISL1 and HNK1. Disease hallmarks were determined using genotyping, qPCR, immunocytochemistry and Western blot.
Results: We generated large-size, proprioceptive and mechanoreceptive neurons as well as small-size, nociceptive neurons by modulating the treatment with small molecules (Notch inhibition, WNT agonists) and neurotrophic factors (NT3, NGF and BDNF). By following the expression of specific marker sets in different conditions, we were able to characterize different neuronal subtypes. After 10 days of NT3 and BDNF treatment, PSNs expressed PRPH, HNK1, BRN3A, ISL1, NGN2, P75NTR, TRKB, TRKC and vGLUT2, indicating a proprioceptive subtype.
With these peripheral sensory neurons at hand, we generated a more appropriate disease model for Friedreich ataxia, which will help us to get more insight into cell-type specific pathophysiological processes.

Neuronal response to carbachol stimulation is altered in the medial prefrontal cortex of mice with neuropathic pain

Miodrag Mitric[1], Kai Kummer[1], Michaela Kress[1]

Division of Physiology, Medical University of Innsbruck, Innsbruck, Austria

miodrag.mitric@i-med.ac.at

Aims: Several lines of evidence have shown that the medial prefrontal cortex (mPFC) plays a role in the modulation of pain processing. Disruption of cholinergic transmission to mPFC has been associated with the impairment of cognitive functions, comorbidity frequently present in chronic pain patients. Therefore, we tested the effect of cholinergic stimulation on neuronal activity in acute mPFC slices obtained from spared nerve injury (SNI) and sham-operated mice.
Methods: Using in vitro multielectrode arrays (Multi Channel Systems) we recorded the effect of carbachol (CCh) application on neuronal activity in acute brain slices of male C57BL/6J mice 7 days after surgery. After five minutes of baseline recording, slices were superfused twice with 1, 5, 25, 50 or 100 µM CCh, each time followed by a washout phase.
Results: We found that CCh induced dose-dependent firing of neurons in all recorded mPFC regions (i.e., prelimbic, infralimbic and anterior cingulate cortices). The overall amount of electrodes detecting spikes was increased from 6% before to 45% after 100 µM CCh stimulation. There was no difference in baseline activity between the two treatment groups. However, spiking frequency induced by 50 and 100 µM CCh was significantly lower in the SNI group compared to the control. In addition, a strong decrease in the response was noted upon repetitive applications.
Conclusions: These results suggest that neuropathic pain induces changes in cholinergic transmission in mPFC and address a need for further investigation of underlying mechanisms.

Poster # 61 neuroscience

A new paradigm to model non-motor symptoms of Parkinson's disease in mice

Sara Ferrazzo, Claudia Schmuckermair, Francesco Ferraguti

Inst. for Pharmacology, Medical University of Innsbruck, Austria

sara.ferrazzo@i-med.ac.at

Parkinson's disease (PD) is classically considered as a movement disorder resulting from the loss of dopaminergic (DA) neurons in the substantia nigra (SNc). However, considerable evidence suggests that non-motor symptoms, including pathological fear and anxiety, predate the emergence of motor impairment in PD patients. Motor symptoms become typically apparent only after 60-70% loss of nigral DA neurons, likely due to the large DA receptor reserve of the striatum. Psychiatric symptoms, including fear and anxiety, may depend on a lower capacity of the limbic system to adapt to DA denervation compared to the striatum.

To model these non-motor symptoms in mice, we stereotactically delivered the neurotoxin 6-hydroxydopamine (6-OHDA) into the basal amygdala (BA) of C57Bl6/J mice to selectively lesion midbrain DA neurons innervating the amygdala and associated limbic structures. Stereological analysis of the two main mesencephalic DA nuclei, the SNc and ventral tegmental area (VTA), revealed that intra-BA 6-OHDA injections resulted in a predominant cell loss in the SNc, questioning the widely accepted fact that the DAergic innervation of the amygdaloid complex is mainly derived from the VTA.

The DAergic denervation compromised most amygdaloid substructures (except the central nucleus) as well as the ventral hippocampus but not the prefrontal cortex. When 6-OHDA-lesioned mice were tested for motor function no overt phenotype was observed. On the other hand, the lack of DAergic innervation in these structures resulted in an anxiogenic phenotype, whereas fear memory acquisition was unaffected.

Taken together, our results indicate that our mouse model reliably recapitulates several characteristics of early phase Parkinson's disease including (i) a limited degeneration of midbrain dopaminergic neurons, (ii) lack of motor symptoms and (iii) enhanced anxiety-like behaviour. Moreover, we reveal an unexpected participation of SNc neurons in the DAergic innervation of the amygaloid complex. Further studies have to clarify the exact origin of DAergic inputs to the functionally distinct amygdaloid subnuclei and their contribution to anxiety.

Modulation of Fibroblast Growth Factor receptor 1 signalling by Nogo-66 receptor 1 in sensory neurons

neuroscience Poster # 62

Barbara Fogli, Letizia Marvaldi, Barbara Hausott, Lars Klimaschewski

Division of Neuroanatomy, Department of Anatomy and Histology, Medical University of Innsbruck, Austria

barbara.fogli@i-med.ac.at

The Fibroblast Growth Factor (FGF) family comprises 23 members originally identified as potent mitogens for different cell types. The majority of these factors is secreted and bind to four types of tyrosine kinase receptors (FGFR1-4) leading to their dimerization and to the auto-phosphorylation of their intracellular domains which induces the activation of three main signaling pathways: the Ras/MAPK-, PI3K/Akt- and the PLCγ-Ca2+ pathway. In the nervous system, FGF-2 and its high-affinity receptors, FGFR1 and FGFR2, are constitutively expressed in adult dorsal root ganglion (DRG) sensory neurons and in the peripheral nerve where, after a lesion, FGF-2 up-regulation promotes their survival and enhances neurite outgrowth.

The effect of FGFR1 activation may involve its interaction with other receptors such as Nogo-66 receptor 1 (NgR1), a negative regulator of neuronal growth. In fact, the ectopic over-expression of NgR1 blocks FGF-2-induced differentiation of PC12 cells and axonal branching in primary cortical neurons. Even if a direct interaction of FGFR1 and NgR1 has not been described, a high-affinity binding of FGF-2 to NgR1 was demonstrated (Lee et al., 2008).

We aim to investigate by which mechanisms NgR1 modulates FGFR1 signalling in sensory neurons. Our hypotheses rely on a possible direct interaction between the two receptors or in the ability of NgR1, dependently or independently of FGF-2 binding, of modulating FGFR intracellular signalling and trafficking.

Our preliminary experiments show a stronger FGF-2-dependent increase in axon outgrowth in NgR1 knock-out DRG neurons compared to wild type neurons plated on laminin, a growth promoting substrate. This effect is specifically mediated by FGF-2 binding to FGFR, since it is reversed by PD173074, a specific inhibitor of FGFRs.

In conclusion, we could demonstrate that NgR1 acts as a negative regulator of FGFR signalling in adult sensory neurons culture.

Cholinergic neurodegeneration in an Alzheimer mouse model overexpressing amyloid-precursor protein with the Swedish-Dutch-Iowa mutations

Bettina Maria Foidl[1], Patricia Do-Dinh[1], Bianca Hutter-Schmid[1], Harald Bliem[2], Christian Humpel[1]

[1]Laboratory of Psychiatry and Exp. Alzheimer's Research, Department of Psychiatry and Psychotherapy, Medical University of Innsbruck, Austria
[2]Institute of Psychology, University Innsbruck

bettina.foidl@i-med.ac.at

Alzheimer's disease (AD) is a chronic neurodegenerative disorder that is mainly characterized by beta-amyloid (A) plaque deposition, Tau pathology and dysfunction of the cholinergic system. The aim of the present study was to examine (1) anxiety and cognition, (2) A plaque deposition and (3) degeneration of cholinergic neurons in the nucleus basalis of Meynert (nbM) and cortical cholinergic innervation in an Alzheimer mouse model (APP_SweDI; overexpressing amyloid precursor protein (APP) with the Swedish K670N/M671L, Dutch E693Q, and Iowa D694N mutation). Our results show that 12 month old APP_SweDI mice were more anxious and impaired in memory. A high number of A plaques was already visible at the age of 6 month and increased with age. There was a significant decrease of cholinergic neurons in the transgenic mouse model in comparison to the wild-type mice, identified by immunohistochemistry against both choline acetyltransferase (ChAT) and p75 neurotrophin receptor as well as by in situ hybridization. Moreover, a significant decrease of cortical cholinergic fiber density was found in the transgenic mice compared to the wild-type. In the cerebral cortex of APP_SweDI mice, swollen cholinergic varicosities were seen in the vicinity of A plaques. Our results show that the APP_SweDI mouse model expresses A plaques accompanied by extensive cholinergic neurodegeneration and cognitive impairment.

Establishment Of Predictive Validity In The PLP aSYN Mouse Model Utilizing Spectral And Behavioral Parameters Of The EEG

Lorenz Härtner[1], T. Keil[1], M. Kreuzer[2], E.M Fritz[1], G. Wenning[3], N. Stefanova[3], T. Fenzl[1]

[1]Department of Pharmacology and Toxicology, University Innsbruck, Austria
[2]Department of Anesthesiology, Emory University, Atlanta USA
[3]Department for Neurology, Medical University Innsbruck, Austria

lorenz.haertner@student.uibk.ac.at

Multiple System Atrophy (MSA) is a rapidly progressing, fatal neurodegenerative disease.
Clinical symptoms include parkinsonism, cerebellar ataxia, autonomic failure and sleep symptoms. Sleep related symptoms, such as rapid eye movement sleep behavior disorder (RBD), breathing disorders and restless legs syndrome, are very common in MSA patients and precede MSA diagnosis.
The presence of similar sleep related symptoms should be expected in an animal model with extensive behavioral phenotype, such as the PLP α-SYN model, increasing face validity. Furthermore, differences in an easily accessible parameter, such as longitudinal EEG recordings, may prove suitable as a biomarker for disease onset. Additionally, an ideal animal model should also provide predictive validity.
As sleep/wake behavior of the otherwise well characterized PLP α-SYN mouse model (MSA mouse) is still unknown, we performed chronical EEG studies in freely behaving MSA and C57BL\6 mice.
The study included young MSA mice without motor symptoms, aged MSA mice showing an extensive behavioral phenotype, and aged matched C57BL\6 N controls.
Multiple differences in the sleeping behavior could be detected: Young MSA mice showed an increased rapid eye movement sleep (REMS) during their inactive period. Increased spectral power of the EEG during wakefulness and REMS could also be detected, compared to controls. The finding of increased spectral power provides a striking resemblance of studies performed in humans. MSA mice showed also age dependent skeletal muscle tone during REMS, which is one of the core symptoms of RBD.
Our results clearly show the presence of RBD-like symptoms in the PLP αSYN mouse model. As it is an early symptom occurring in a vast majority of MSA patients, this finding strongly increases the face validity of the mouse model. Increased spectral power and increased REMS only occur in young MSA mice. No such findings could be detected in the control group. As these parameters are relatively easy accessible in the freely behaving animal, we suggest them for future basic points in predictive validity.

NPY and Y2R in Adult Neurogenesis and Fear learning

Birgit A Hörmer[1], Dilip Verma[1], Elisabeth Gasser[1], Herbert Herzog[2], Ramon O Tasan[1]

[1]Department of Pharmacology, Medical University Innsbruck, Innsbruck, Austria
[2]Neuroscience Research Program, Garvan Institute of Medical Research, Sydney, Australia

birgit.hoermer@i-med.ac.at

Neuropeptide Y (NPY) is an anxiolytic neuromodulator promoting hippocampal adult neurogenesis, predominantly by activating Y1 receptors. The role of pre-synaptic Y2 receptors (Y2R) in these processes, however, remains to be determined. Interestingly, Y2R are expressed on mature granule cells and possibly also on neurons located in the dentate hilus mediating there inhibition of NPY, GABA or glutamate release. The aim of the present study is to investigate the role of Y2R in hippocampal adult neurogenesis and the effects on hippocampus mediated fear learning. These questions will be addressed by combining Pavlovian fear conditioning with birth dating of newly born neurons. First, we investigated the anatomical expression patterns of NPY and Y2R in the neurogenic niche of the adult hippocampus. We found that NPY is not only expressed by somatostatin-neurons but also by those parvalbumin-positive basket cells that are close to the subgranular zone. Y2R are expressed by mature granule cells and by calretinin-neurons, the latter probably representing hilar mossy cells. Secondly, we subjected Y2KO mice to fear learning paradigms that are dependent on the availability of newly born neurons. We discovered that Y2KO mice are unable to differentiate between similar fear contexts in a pattern separation paradigm. In line, we found that Y2KO mice lack the temporal precision and stimulus specific expression of freezing behavior in trace fear conditioning, suggesting a generalization of fear. However, when Y2R was reintroduced in the dorsal dentate gyrus, freezing behavior was significantly reduced and temporal precision was largely restored. These data indicate that Y2R are crucial for differentiating similar fear contexts and that absence of Y2R may result in fear generalization, a hallmark of anxiety disorders. Furthermore, the presence of Y2 receptors on mossy cell terminals may provide a crucial link for adapting adult neurogenesis to network activity and control of fear behavior.

Platelet-derived growth factor receptor-beta is differentially regulated in primary mouse pericytes and brain slices

Bianca Hutter-Schmid, Christian Humpel

Laboratory of Psychiatry and Exp. Alzheimer's Research, Department of Psychiatry and Psychotherapy, Medical University of Innsbruck, Austria

bianca.hutter-schmid@i-med.ac.at

Pericytes are perivascular cells and have heterogenous roles in the brain, such as controlling blood flow and entry of immune cells or regulating the blood-brain barrier. Platelet-derived growth factor (PDGF) receptor-beta (PDGFRβ) is highly expressed in pericytes, representing the most selective biomarker. The aim of the present study was to culture primary mouse pericytes and to determine the expression pattern by Western Blot as well as immunostainings. We will study the effects of different exogenous stimuli (such as transforming growth factor-β (TGFβ1), PDGF-BB, oxygendeprivation, beta-amyloid or serumfree conditions) on the different pericyte markers. Using Western Blot analyses, we show that PDGFRβ is selectively expressed in pericytes as a 160 kDa protein. Nestin, although not exclusively specific, is also expressed by pericytes, but markedly downregulated under serum-free conditions. PDGF-BB and oxygen-deprivation dramatically reduced PDGFRβ expression, while TGFβ1 increased its expression. The expression of PDGFRβ was intracellular as shown by confocal microscopy. Using Western Blot analyses, we demonstrate that pericytes also contain a 100 kDa PDGFRβ protein. However, in contrast to cortex brain slices, pericytes do not express a phosphorylated (Y740) isoform. Interestingly, PDGF-BB markedly reduced the 160 kDa isoform of PDGFRβ. In conclusion, our data show a detailed expression of different forms of PDGFRβ in primary pericytes, which is different to brain slices. However, we suggest that PDGFRβ is a highly selective marker for pericytes.

Neurosensory development in the human foetal vestibular end organs

Lejo Johnson Chacko[1], EJ Pechriggl[2], H Fritsch[2], H Rask-Andersen[4], MJF Blumer[2], R Glueckert[1,3], A Schrott-Fischer[1]

[1]Department of Otolaryngology, Medical University of Innsbruck, Innsbruck, Austria
[2]Department of Anatomy, Histology and Embryology, Division of Clinical and Functional Anatomy, Medical University of Innsbruck, Innsbruck, Austria
[3]University Clinics Innsbruck, Tiroler Landeskrankenanstalten, Innsbruck, Austria
[4]Departments of Otolaryngology, Uppsala University Hospital, Uppsala, Sweden

lejo.johnson@i-med.ac.at

Previous investigations on the assemblage of the neuronal network of the human vestibular organ are sparse, seemingly, due to the onerous task of accessing foetal specimens. This insufficiency has caused most investigations to be performed on murine models. Using immunohistochemical and transmission electron microscopic techniques, we scrutinized an uninterrupted series of unique specimens from gestational week 8 to 12. We were able to demonstrate the early maturation of the vestibular sensory epithelia as indicated by the expression of the transcription factor PAX2 and the glycoprotein synaptophysin. Staining for these is confined to the sub-sensory regions in the foetal cochlea at week 12. Intense staining for Beta-III tubulin which is specific for both type I and type II Spiral ganglion neurons (SGNs) in the vestibular end organs from week 8 while the staining is confined to a few cells in the cochlea during this period. Intense pattern of staining was seen for peripherin, which is specific for type II SGN's similar to that seen in the cochlea. Expression for Pax6 was seen for the first time at week 10 among the supporting cells and the mesenchyme underlying the sensory epithelia. It indicated an as yet uncertain role for this transcriptional factor in inner ear development. Unlike that in the foetal cochlea there was an early expression of the transcription factor MAF B noted in the Scarpa ganglion while tyrosine hydroxylase expression is visible unlike that in the foetal cochlea.

The role of calcium channels in acetylcholine receptor pre-patterning during neuromuscular junction development

Nasreen Sultana, Ariane Benedetti, **Mehmet M Kaplan**, Bernhard E Flucher

Department of Physiology and Medical Physics, Innsbruck Medical University, Innsbruck, Austria

mehmet.kaplan@i-med.ac.at

Motor neurons control muscle contraction at cholinergic synapses called neuromuscular junctions (NMJ). To achieve efficient neurotransmission, acetylcholine receptors (AChRs) are densely clustered at the postsynaptic membrane. Mammalian NMJs typically organize within a narrow endplate band located in the center of the muscle fibers. Whereas nerve-derived factors are important for the formation and stabilization of NMJ, the initial formation of AChR clusters in the central endplate band—called AChR pre-patterning—occurs prior to the arrival of the nerve and thus is accomplished by muscle-intrinsic mechanisms. A recent study indicated an essential role of L-type calcium currents in AChR pre-patterning (Chen et al., 2011; Nat. Neurosci. 14:570-7). According to this study, L-type calcium currents through Cav1.1 are required for proper pre-patterning of AChRs independent of the role of Cav1.1 as voltage sensor in EC-coupling. Recently, our laboratory discovered a new embryonic splice variant of Cav1.1 lacking exon 29 (Cav1.1e) (Tuluc et al., 2009; J Biophys. 96:35-44). Since this splice variant is expressed at the critical stage for AChR pre-patterning and, at variance with the adult CaV1.1a splice variant, conducts sizeable calcium currents, we hypothesize that L-type calcium currents through Cav1.1e support AChR pre-patterning. If this is the case, pre-patterning will fail in absence of L-type calcium currents. To test this hypothesis, we studied AChR pre-patterning in embryonic E14.5 diaphragm muscles of several calcium channel mutant mouse models. Two viable knock-in mice: Cav1.1ΔE29 mice, expressing only the well conducting embryonic Cav1.1e variant; and Cav1.1-nc mice, generated in the laboratory of Prof. Manfred Grabner and expressing only non-conducting Cav1.1 channels (Dayal et al., 2014; J Biophys) ; and two lethal mouse models due to failure of EC coupling: dysgenics (mdg) mice, which entirely lack CaV1.1; and dyspedics (RyR-/-) mice, which express CaV1.1 channels but lack the SR release channel. As predicted from the absence or presence of CaV1.1, pre-patterning failed in dysgenic mice, but was intact in Cav1.1ΔE29 and dyspedic mice. Surprisingly however, AChR pre-patterning was also normal also in Cav1.1-nc mice. This result clearly demonstrates that AChR pre-patterning requires CaV1.1, but is independent of calcium influx through this channel. To further elucidate the role of calcium signaling in this process, double knockout mice will be bred and investigated, and the spatial and temporal distribution of the calcium signaling machinery during the critical phase of AChR pre-patterning will be investigated.

The medial prefrontal cortex: a key region involved in the extinction-facilitating effect of L-DOPA in a psychopathological mouse model of impaired fear extinction

Thomas M.V. Keil, S.B. Sartori, V. Maurer, C. Murphy, N. Whittle, N. Singewald

Department of Pharmacology and Toxicology, Institute of Pharmacy and Center for Molecular Biosciences Innsbruck, University of Innsbruck, Innsbruck, Austria

t.keil@student.uibk.ac.at

Despite its success in treating specific anxiety disorders, the effect of exposure-based therapy is restricted by limited duration and occurrence of fear relapse after initial response. Recent evidence suggests that, in healthy subjects, enhancing dopaminergic signalling promotes successful long-term fear extinction, i.e. a main mechanism underlying exposure-based therapy. Here, we exploited the extinction-facilitating potential of the dopamine bioprecursor L-DOPA in extinction-resistant 129S1/SvImJ (S1) mice using a classical cued fear conditioning paradigm. Fear relapse was studied in several test sessions following extinction training. Single systemic application of L-DOPA induced fear extinction in S1 mice. In order to identify the neuronal substrates mediating the observed behavioral effects in S1 mice, we performed dopamine microinjection studies targeting the medial prefrontal cortex (mPFC); a brain region where we have previously shown upregulated dopamine-related genes upon successful fear extinction. The intra-mPFC infusion of dopamine caused constant and pronounced reductions in fear responses during extinction training in S1 mice. In contrast to the rather short-lived effect of systemic L-DOPA treatment, fear extinction persisted up to 10 days after extinction training in S1 mice. Microinfusion of either a selective dopamine D1 or D2 receptor agonist had a smaller effect on fear extinction in S1 mice, suggesting that activation of either both D1 and D2 receptors or of additional dopamine receptors is necessary for mediating the extinction-promoting effects of dopamine in the mPFC. Overall, the present findings imply that, in extinction-resistant subjects, robust fear-extinction memories can be formed by increasing dopaminergic signalling in the mPFC.

Thiazine Red$^+$ platelet inclusions in Cerebral Blood Vessels are first signs in an Alzheimer's Disease mouse model

Kathrin Maria Kniewallner[1], Daniela Wenzel[2], Christian Humpel[1]

[1]Laboratory of Psychiatry and Exp. Alzheimer's Research, Department of Psychiatry and Psychotherapy, Medical University of Innsbruck, Austria
[2]Institute of Physiology I, University of Bonn, Germany

kathrin.kniewallner@i-med.ac.at

There is strong evidence for an association between cerebral vascular diseases and Alzheimer´s disease (AD). Several vascular risk factors may play a role in the deposition of the beta-amyloid (Aβ) peptides in the brain (plaques) and in vessels (cerebral amyloid angiopathy, CAA) in AD. In order to study the development of plaques and the association with brain vessels, we crossbred an Alzheimer mouse model (overexpressing amyloid precusor protein with the Swedish-Dutch-Iowa mutations, APP_SweDI) with mice showing green fluorescent protein (GFP+) vessels (under the FLT-1 promoter; GFP_FLT1). Our data show, that only very few Aβ plaques were seen in young 4 month old mice, focused in the mammillary body and in the lateral septal nucleus. The number of plaques markedly increased with age being most prominent in 12 month old mice. Thiazine Red was used to verify the plaques. Interestingly, several Thiazine Red inclusions were found in GFP+ vessels, but only in 4 month old mice and only in non-perfused mice. These inclusions were verified by Resorufin stainings possibly representing CAA. In order to characterize these inclusions FACS analysis demonstrated that platelets can be specifically stained by Thiazine Red, more pronounced when aggregated. In conclusion, our data show that Thiazine Red+ inclusions representing aggregated platelets are a first pathological sign in AD before plaque development.

Impact of Cav1.4 L-type calcium channels on visual function and circadian activity

Dagmar Knoflach[2], Daniel Üblagger[2], Stefanie Geisler[3], Klaus Schicker[2], Gerald Obermair[3], Daniela Pollak[2], Alexandra Koschak[1]

[1]University of Innsbruck, Institute of Pharmacy, Pharmacology and Toxicology, Innsbruck
[2]Medical University Vienna, Department of Neurophysiology and Pharmacology, Vienna
[3]Medical University Innsbruck, Division of Physiology, Innsbruck

d.knoflach@student.uibk.ac.at

Cav1.4 L-type calcium channels are abundantly expressed in photoreceptors at their synaptic terminals where they control neurotransmitter release. We previously showed that the introduction of a Cav1.4 gain-of-function mutation (Cav1.4-IT) in mice severely affected their retinal morphology and also changed the sensitivity to light. For circadian rhythm light is an important zeitgeber and the suprachiasmatic nucleus (SCN) functions as central pacemaker. The SCN receives light signals from the retina through the retinohypothalamic tract and directly regulates melatonin biosynthesis in the pineal gland, which is the executive endocrine organ releasing melatonin during night. Interestingly the expression profile of L-type calcium channels in the pineal gland, evaluated by Taqman qRT-PCR analysis, was comparable to the one retina. In both tissues Cav1.4 α1 subunit and the auxiliary subunits β2 and α2δ-4 were the main subtypes forming the calcium channel complex in wild type (WT) animals. To evaluate the impact of Cav1.4 channel activity we tested the circadian rhythm of Cav1.4-IT and WT mice in a behavioural paradigm. The mice were placed for 13 days in light:dark cycle (LD; 12:12 hours) followed by 24 hours darkness (DD), the so called free-running period, for 10 days. After 10 days the mice were exposed to a brief light impulse (30 min, 300 lux) at circadian time 16 (which is 4h after activity onset) to induce a phase shift response. The DD cycle was then maintained for another 10 days. Under LD condition, Cav1.4-IT mice showed no change in activity compared to WT. However during the free-running period, in which the circadian rhythm is not influenced by external zeitgeber, the activity onset in Cav1.4-IT was shifted to the left by 27 minutes. The phase shift response was comparable in Cav1.4-IT and WT mice (light-induced phase delay at CT 16 [h]: WT: -2.07 ± 1.83, n =11; Cav1.4-IT: -1.51 ± 0.83, n = 11; mean ± SD). We further investigated whether the circadian behavioral phenotype could be explained at the molecular level by analyzing clock gene expression in the SCN. The expression levels of the key clock genes were analyzed by qRT-PCR. Expression of all clock genes investigated in SYBR Green based assays (CLOCK, BMAL1, PER1-3, CRY1-2, Reverbα-β, RorA,B and G and Npas2) was unchanged in Cav1.4-IT compared to WT. Together we suggest that changes in Cav1.4 channel activity do not only change visual function but might also affect circadian activity.

Social interaction reward decreases p38 activation in the nucleus accumbens shell of rats

Cristina Lemos[1], Ahmad Salti[1], Kai K. Kummer[2], Chinmaya Sadangi[1], Georg Dechant[3], Alois Saria[1], Rana El Rawas[1]

[1]Experimental Psychiatry Unit, Medical University of Innsbruck, Innsbruck, Austria
[2]Division of Physiology, Medical University of Innsbruck, Innsbruck, Austria
[3]Institute for Neuroscience, Medical University of Innsbruck, Innsbruck, Austria

ana-cristina.figueiredo@i-med.ac.at

We have previously shown that animals acquired robust conditioned place preference (CPP) to either social interaction alone or cocaine alone. Recently it has been reported that drugs of abuse abnormally activated p38, a member of mitogen-activated protein kinase family, in the nucleus accumbens. In this study, we aimed to investigate the expression of the activated form of p38 (pp38) in the nucleus accumbens shell and core of rats expressing either cocaine CPP or social interaction CPP 1 h, 2 h and 24 h after the CPP test. We hypothesized that cocaine CPP will increase pp38 in the nucleus accumbens shell/core as compared to social interaction CPP. Surprisingly, we found that 24 h after social interaction CPP, pp38 neuronal levels were decreased in the nucleus accumbens shell to the level of naïve rats. Control saline rats that received saline in both compartments of the CPP apparatus and cocaine CPP rats showed similar enhanced p38 activation as compared to naïve and social interaction CPP rats. We also found that the percentage of neurons expressing dopaminergic receptor D2R and pp38 was also decreased in the shell of the nucleus accumbens of social interaction CPP rats as compared to controls. Given the emerging role of p38 in stress/anxiety behaviors, these results suggest that (1) social interaction reward has anti-stress effects; (2) cocaine conditioning per se does not affect p38 activation and that (3) marginal stress is sufficient to induce p38 activation in the shell of the nucleus accumbens.

Proangiogenic neuropeptides in experimental choroidal neovascularisation and comparison of two different techniques for CNV volume measurement

Yvonne Nowosielski, G. Haas, C. Seifarth, J. Troger, N.E. Bechrakis

Department for Opthalmology and Optometry at the Medical University of Innsbruck

yvonne.nowosielski@i-med.ac.at

Background: Wet age-related macular degeneration (AMD) is the most frequent reason for vision loss in elderly patients in the industrialised world. It is characterised by the formation of choroidal neovascularisations (CNVs) which sprout from the choroid into the subretinal space causing macula edema and/or bleeding resulting in central vision loss. Vascular endothelial growth factor is known to be the major involved growth factor in choroidal angiogenesis. In this study we aimed to evaluate firstly whether the neuropeptides substance P (SP) and neuropeptide Y (NPY), which act in a proangiogenic manner with a potency similar to that of VEGF, contribute to the pathogenesis of CNVs. Secondly we evaluated two different techniques for CNV volume measurement: a rather new in vivo measurement technique using spectral-domain optical coherence tomography (SD-OCT) and the commonly used ex vivo flatmount technique.

Methods: Volumes of laser- induced CNVs were obtained on day four after laser application. Each mouse eye was then analysed in vivo by SD-OCT. The volume was obtained by summing up the CNV area per slice and multiplying it with the slice thickness using Image J-software. For the ex vivo method mice were sacrificed, eyes enucleated, flatmounted and stained for von Willebrand factor. Volumes were obtained by analysing z-stacks taken by a laser scanning confocal microscope with Imaris software. Seven C57bl6/N wildtype mice were compared to five Y2- (NPY2-receptor) and four Nk1- (SP-receptor) knockout mice of the same background. Differences in the CNV volume of single spots between wildtype and knockout mice were analysed with Mann-Whitney U test (MWU). Five wildtype, five Y2-knockout and four Nk1-knockout mice with C57bl6/N background were used for comparison of the two methods. Pearson correlation was performed on mean volumes of CNV spots per mouse. Mice were between eight and twelve weeks old. $P < 0.05$ was considered to be significant.

Results: Comparing CNV spots in wildtype (n=14) with Y2-knockout (n=22) and Nk1-knockout mice (n=20) no difference in mean OCT volume could be detected (0.38 vs 0.36 vs 0.29 mm3, p=0.360, p=0.204, respectively, MWU). No difference in mean volume could be detected either using choroidal flatmounts for volume measurements (0.00028 vs 0.00024 vs 0.00036 mm3, p=0.309, p=0.133, respectively, MWU). In comparison the two methods used to obtain CNV volumes showed a significant correlation (R=0.543, p=0.045, n=14 mice).

Conclusion: Despite the fact that there was no significant difference between CNV volume comparing knockout to wildtype mice, knockout mice generally still showed reduced volumes. Therefore we decided to add further analyses including a higher number of mice. OCT is useful as a non-invasive tool for the in vivo measurement of laser-induced CNVs and provides even more detailed information on the angiogenic process. Furthermore in vivo methods help to reduce the number of mice to be sacrificed.

Epitope Specificity of human MOG Antibodies and their Pathogenic Effect in Neuroinflammatory Demyelinating Diseases

Patrick Peschl[1], Kathrin Schanda[1],Romana Höftberger[2], Albert Saiz[3], Monika Bradl[4], Bleranda Zeka[4], Kevin Rostasy[5], Thomas Berger[1], Markus Reindl[1]

[1]Clinical Department of Neurology, Medical University of Innsbruck, Austria.
[2]Institute of Neurology, Medical University of Vienna, Austria
[3]Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain
[4]Department for Neuroimmunology, Center for Brain Research, Medical University Vienna, Austria
[5]Department of Pediatric Neurology, Children´s Hospital Datteln, University Witten/Herdecke, Datteln, Germany

patrick.peschl@i-med.ac.at

Introduction: The myelin oligodendrocyte glycoprotein (MOG) is an important target of autoantibodies associated with inflammatory demyelinating diseases like acute disseminated encephalomyelitis (ADEM), neuromyelitis optica spectrum disorders (NMOSD) or clinical isolated syndromes (CIS). Although experimental studies suggest that antibodies against MOG can augment demyelination in rodents and primates, a definite proof of the pathogenic role of human MOG antibodies is still missing. Since a previous study from our group indicated that only a subset of human MOG antibodies recognize rodent MOG, we were interested to identify MOG antibody seropositive patients which are also reactive to rat and mouse MOG, as a prerequisite to investigate the effect of MOG antibodies in-vivo.
Methods: 100 high titer human MOG positive serum samples from 73 patients were screened for their reactivity to mouse or rat MOG transfected HEK293A cells by a live immunofluorescence cell-based assay (CBA). Serum samples from 35 MOG seropositive patients (14 positive against rat MOG) were further analyzed via immunohistochemistry (IHC) on rat brain slices. To test whether this binding was specific we preabsorbed serum samples with MOG transfected cells. The effect of purified IgG from several MOG positive patients is investigated in an in-vivo rat MBP T-cell mediated experimental autoimmune encephalitis model.
Results: Our present data show that only a subset of human MOG-antibodies is reactive to either mouse (71%) or rat (26%) MOG transfected HEK293A cells. In a next step the binding of serum samples of 35 MOG antibody positive patients was analyzed with IHC on rat brain slices. 9/35 (25%) of the samples which were also positive for rat MOG showed a specific tissue staining. Not surprisingly, these samples showed significant higher antibody titer to rat MOG compared to samples with negative staining. However, we could not detect a statistically significant association with clinical and immunological data. Currently, we investigate whether MOG antibodies are pathogenic in-vivo in a rodent animal model.
Conclusion: These data show that only a subset of human MOG antibody positive serum samples is also reactive to rat or mouse MOG. Additionally, sera with a higher MOG titer to rodent MOG have a better binding efficiency on rat brain tissue. With this finding we are now able to further investigate potential pathogenic effects of selected MOG positive serum samples on a suitable animal model in vivo.

Dyadic social interaction of C57BL/6 mice vs interaction with a toy mouse

Barbara S Pinheiro[1], Simon S Seidl[2], Eva Habazettl[2], Bernadette E Gruber[2], Tanja Bregolin[1], Gerald Zernig[1,2]

[1]Experimental Psychiatry Unit, Department of Psychiatry I, Medical University of Innsbruck,
[2]Department of Psychology, University of Innsbruck, Innsbruck, Austria

barbara.pinheiro@i-med.ac.at

Impaired social interaction is a hallmark symptom of many psychiatric diseases, including dependence syndromes. Helping the addict reorient her/his behavior away from the drug of abuse toward social interaction would be of considerable therapeutic benefit. To study the neural basis of such a reorientation, we have previously developed several animal models in which the attractiveness of a dyadic social interaction (DSI) can be directly compared to that of cocaine as a prototypical drug of abuse.
Aim: To compare the ability of DSI with a live mouse to that of an interaction with an inanimate object resembling a mouse (i.e., a 'toy mouse'; toy mouse interaction, TMI) to produce conditioned place preference (CPP) or conditioned place aversion (CPA), using two different mice substrains.
Methods: Two different substrains of C57BL/6 mice, Jackson (C57BL/6J) and NIH (C57BL/6N), were place preference conditioned for DSI (Zernig and Pinheiro 2015, Behavioural Pharmacology 26, 580) or TMI.
Results and Discussion: DSI with a live mouse produced CPP, whereas TMI lead to CPA, but only in Jackson substrain. In the NIH substrain, both DSI and TMI produced individual aversion in more than 50% of the tested mice. The present findings indicate that DSI with a live mouse produces CPP to a greater degree than the TMI and that certain substrain differences with respect to CPP/A to DSI do exist. These differences have to be considered when choosing a proper mouse substrain model for investigating the neural basis of dyadic social interaction reward vs drug reward.

Meninges harbor cells expressing neural precursor markers during development and adulthood

Annachiara Pino[1], F. Bifari[2], V. Berton[1], M. Kusalo[1], G. Malpeli[3], M. Di Chio[1], E. Bersan[1], E. Amato[3], A. Scarpa[3], M. Krampera[2], G. Fumagalli[1], I. Decimo[1]

[1]Department of Diagnostics and Public Health, Section of Pharmacology, University of Verona, Verona, Italy
[2]Department of Medicine, Stem Cell Research Laboratory, Section of Hematology, University of Verona, Verona, Italy
[3]Department of Pathology, Section of Pathological Anatomy, University of Verona, Verona, Italy

annachiara.pino@univr.it

Brain and skull developments are tightly synchronized, allowing the cranial bones to dynamically adapt to the brain shape. Meninges are the stromal tissue that represents the physical interface between brain and skull and they produce trophic signals necessary for normal corticogenesis and bone development. Different cell populations have been described in meninges, including cells that can function as endosteum of the cranial vault. Recently, we and other groups described the presence in meninges of a cell population endowed with neural differentiation potential in vitro and, after transplantation, in vivo. However, whether meninges may be a niche for neural progenitor cells during embryonic development to adulthood is not known.

With this study we provided the first description of the distribution of neural precursor markers in rat meninges, during development up to adulthood. We described that meninges share common properties with the classical neural stem cell niche: i) meninges are unexpected highly proliferative tissue; ii) they contain cells expressing neural precursor markers such as nestin, vimentin, SOX2 and DCX and iii) meningeal tissue is enriched with extracellular matrix components (fractones) known to bind and concentrate growth factors.

This study provides a new and accurate description of the molecular and cellular aspects of meninges related to its newly identified function of niche for neural progenitor/stem cells. We add to previous information the notion that this niche is indeed present and potentially active at all stages of development and in adult life as well. The identification of receptors for trophic factors, of ECM components and chemotactic factors known to be involved in homing, movement, proliferation and differentiation of progenitor cells strengthens the idea that the niche function of meninges is not limited to conditions associated to diseases, such as injury or ischemia. This study underlines the importance of meninges as a potential niche for endogenous precursor cells during development and in adulthood in physiological conditions.

Progression of microglial activation and neuroinflammatory responses in a transgenic mouse model of multiple system atrophy

Violetta Refolo[1], Serena Venezia[1], Gregor K. Wenning[1], Marina Romero-Ramos[2], Nadia Stefanova[1]

[1]Department of Neurology, Innsbruck Medical University, Innsbruck, Austria
[2]Department of Biomedicine, Aarhus University, Aarhus, Denmark

violetta.refolo@i-med.ac.at

Objective: Our aim was to characterize the progression of microglial activation during disease progression in a transgenic mouse model of multiple system atrophy (MSA).

Background: Microglial cells have been demonstrated to play a pivotal role in MSA pathogenesis, in particular in the neuroinflammatory processes involved in the disease progression. Furthermore, it has become clear that the way microglia act in this context cannot be defined as entirely positive or detrimental, as they show both sides of the coin, and are therefore extremely interesting actors during the disease development.

Methods: For the analysis we used transgenic mice overexpressing α-synuclein in oligodendrocytes under the proteolipid protein promoter (MSA mice) and wild type controls at the age of two, five and fifteen months. We performed morphological characterization of microglia during stereological analysis of different brain areas which are relevant to MSA, according to a rating scale developed by Sanchez-Guajardo et al. (2010). This scale allows the subdivision of microglia in four different forms (A, B, C and D), characterized by increasing levels of activation (from resting microglia to completely activated). We also used a ProcartaPlex Multiplex immunoassay to measure cytokine and chemokine levels in the brain and plasma of the same experimental groups. Two-way ANOVA with variables age and genotype was used for the statistical analysis.

Results: Preliminary data show an increased presence of activated microglia in older MSA mice as compared to both younger MSA mice and control animals of the same age. Furthermore, the levels of specific cytokines and chemokines in the brain change significantly with ageing in MSA mice in contrast to healthy controls and may contribute to the pathogenesis of the disease.

Conclusions: Our findings suggest that there is an increase in the neuroiflammatory state in MSA mice that parallels the progression of the disease. The results of this study will serve future identification of therapeutic targets related to neuroinflammation in MSA.

Connectivity and immunohistochemical characterization of VIP+ interneurons in the distinct subdivisions of the basolateral complex of the mouse amygdala

Thomas Rhomberg[1], Enrica Paradiso[1], Ramon Tasan[1], Christian Kremser[2], Francesco Ferraguti[1]

[1]Department of Pharmacology, Medical University of Innsbruck, Innsbruck Austria
[2]Department of Radiology, Medical University of Innsbruck, Innsbruck Austria

thomas.rhomberg@student.i-med.ac.at

Fear and anxiety disorders are amongst the most prevalent psychiatric disorders worldwide. Our understanding of their pathophysiology is still very limited. A central role in the neural pathways of fear learning is played by the amygdaloid complex, which is composed of several neurochemically and cytologically distinct nuclei. The lateral, basal and basomedial nuclei, which consist of further anatomically and functionally relevant subdivisions, form the basolateral complex (BLA). The BLA contains roughly 80% glutamatergic output principal neurons, whereas the remaining 20% are GABAergic interneurons, which tightly regulate the activity of the principal neurons. These interneurons can be classified into several functionally distinct and non-redundant subgroups based on: firing activity in vivo, axonal projections and molecular fingerprint (e.g. expression of calcium-binding proteins and neuropeptides). Most of the distinct classes of interneuron directly form inhibitory synapses with principal neurons. However, a few subgroups, which express calretinin (CR) and/or the vasoactive intestinal polypeptide (VIP), have been postulated to have as their primary post-synaptic target other interneurons, similar to their counterpart in the hippocampus and neocortex. An interneuron-selective connectivity suggests a disinhibitory function on principal cells, thus facilitating their activity. However the precise functional role of VIP+/CR+ interneurons in emotional-related processes remains entirely unknown. In this project, we have determined the density of VIP+ and CR+ interneurons in each subdivision of the mouse BLA, as well as the proportion of interneurons colocalizing the two molecules. To precisely determine the cell density, the shrinkage of the brain due to fixation and processing was determined by comparative in-vivo and in-vitro magnetic resonance imaging (MRI). Our results show that the posterior subdivision of the basal nucleus (BLp) contains the highest density of VIP+ neurons (1347 cells/mm^3), whereas the dorsolateral subdivision of the lateral nucleus (LaDL) has the lowest density (769 cells/mm^3). Moreover, our findings indicate unexpectedly that the amount of VIP+ neurons does not correlate with the number of VIP+ terminals in the BLA. The coexpression of VIP and CR varied among the distinct subdivisions of the BLA. VIP+ neurons in the BLp contained the lowest % of CR coexpression (42%), whereas all other subdivisions showed a higher degree of colocalization, ranging from 48% in the anterior subdivision of the basal nucleus (BLa) to 68% in the LaVM. In addition, we revealed the colocalization degree of VIP and cholecystokinin (CCK) in the BLA of mice. We could not find a significant difference among the distinct subdivisions and the amount of VIP neurons colocalizing with CCK is much lower (2% - 12%) than previously reported in rat tissue. In a further attempt to reveal the connectivity of VIP+ neurons, we demonstrate that VIP+ interneurons are directly innervated by putative thalamic and cortical afferents as indicated by their expression of the vesicular glutamate transporter (VGluT) 1 or 2, respectively.

The role of the Cav1.4 L-type calcium channels in sleep regulation

L-type calcium channels (LTCCs) are connected to diverse body functions such as muscle contraction, hormone and neurotransmitter release, cardiac pace-making as well as learning and memory. At release sites in mammalian photoreceptors Cav1.4 LTCCs have an impact on the signal transduction pathway of light stimuli from the retina to the suprachiasmatic nucleus (SCN). The SCN controls the production and release of melatonin in the pineal gland. This hormone transfers the daily cycle of light and darkness to the internal control of sleep and wakefulness. A number of X-linked visual disorders (i.e. X—linked retinal disorder, such as incomplete Congenital Stationary Night Blindness) is connected to mutations in the CACNA1F gene, which encodes the pore-forming alpha1 subunit of the Cav1.4 LTCCs.
The aim of this study was to evaluate the role of the Cav1.4 LTCCs in the control of sleep/wake regulation as part of the circadian rhythm in mice. For that we performed chronic electroencephalogram (EEG) and electromyogram (EMG) recordings in a novel Cav1.4 mouse line that includes the point mutation Cav1.4-I745T (IT) and control animals (WT). We analysed the circadian sleep-wake behaviour, including vigilance states WAKE, non-rapid eye movement sleep (NREMS) and rapid eye movement sleep (REMS). Our results clearly showed that in IT mice the sleep onset was synchronised to the light stimuli, but was delayed for roughly 14 minutes, compared to WT. IT mice showed also increased wakefulness during the transition from darkness to light. We further detected increased NREMS during the transition from light to darkness.
The Cav1.4-I745T mutation may be responsible for delayed sleep onset and sleep termination in IT mice, which could serve as a predictive validity tool in future studies on X-linked visual disorders in patients. Given the relationship that sleep disturbances are an comorbidity factor to autism disorders, epilepsy and other psychic disorders, the Cav1.4-I745T mutation may also increase the susceptibility for these diseases.

Alina Richter[1], E.M. Fritz[1], M. Kreuzer[2], A. Koschak[1], T. Fenzl[1]

[1]Department of Pharmacology and Toxicology, University Innsbruck, Austria
[2]Department of Anesthesiology, Emory University, Atlanta USA

alina.richter@student.uibk.ac.at

Poster # 81 neuroscience

The role of microglia/myeloid system in innate anxiety and depression

Sinead Rooney[1], A. Sah[1], S.A. Wolf[2], S.B. Sartori[1], H. Kettenmann[2], N. Singewald[1]

[1]Department of Pharmacology and Toxicology, Center for Chemistry and Biomedicine, University of Innsbruck, Innsbruck, Austria
[2]Department of Cellular Neurosciences, Max Delbrück Center for Molecular Medicine, Berlin, Germany

sinead.rooney@student.uibk.ac.at

Patients with comorbid anxiety and depression suffer substantial impairment in daily life and more pronounced treatment-resistance. In recent years, microglia/myeloid cells and gut microbiota have been implicated in psychiatric disorders, but have not been systematically investigated in this particular comorbidity. The current project will investigate whether a treatment-resistant mouse model of innate anxiety and comorbid depressive-like behaviour (HAB), in comparison to a normal anxiety/depression (NAB) control, shows (i) neuroinflammation and microbiome imbalances, (ii) normalization of these pathological events in response to successful treatment, and (iii) behavioural changes in response to microglia/myeloid inhibitors or activators. Preliminary results indicate that microglia/myeloid cells, in the HAB hippocampus, an enhanced expression of ionized calcium-binding adapter molecule (Iba1; indicative of a pro-inflammatory bias) and a significant increase in number of cells positive for Iba1; thus far, neuroinflammation in HABs is proposed by the current evidence. Faecal bacteria composition and blood-derived immune cells of HABs are hypothesized to also show alterations and this analysis is still ongoing. Previous studies have demonstrated that the HAB model displays an insensitivity to the current first-line treatment option, selective serotonin-reuptake inhibitors (SSRIs). Using enriched environment (EE), which has previously been shown to reduce neuroinflammation and to elicit an anxiolytic/antidepressant-like effect in other animal models, we successfully normalized the pathological anxiety behaviour in HABs. Investigation of EE-induced effects on the microglial/myeloid system and gut microbiome in HABs is underway. Interactions between microglia, peripheral immune cells and gut microbiota in the HAB model will be explored, and the future outcome of this study may reveal possible neuroinflammatory mechanisms of treatment-resistance in individuals with comorbid anxiety and depression.

Rejuvenation of the aged brain through leukotriene receptor inhibition: modulation of phagocytic capacity in aged microglia as a potential mode of action?

neuroscience Poster # 82

Johanna B. Schaffenrath[1,2], M. S. Unger[1,2], B. Klein[1,2], L. Aigner[1,2], J. Marschallinger[1,2]

[1]Institute of Molecular Regenerative Medicine, Paracelsus Medical University, Salzburg, Austria
[2]Spinal Cord Injury and Tissue Regeneration Center Salzburg (SCI-TReCS), Salzburg, Austria

j.schaffenrath@pmu.ac.at

Age-related impairments in cognitive functions and the development of dementias such as Alzheimer´s disease correlate with a plethora of structural changes in the aged brain, e.g. reduced levels of neurogenesis, altered neuronal activity, and increased neuroinflammation, in particular microglia activation. Microglia in the aged brain are characterized by an increased production of proinflammatory cytokines and, importantly, by severe impairments in phagocytosis, one of the principal microglia functions.

Recently, elevated CNS levels of leukotrienes, i.e. lipid mediators of inflammation, and of the associated 5-LOX signaling pathway have been shown to contribute to neuroinflammatory processes in the brain and to the development of cognitive declines and dementias.

Indeed, we could demonstrate that inhibition of leukotriene signaling by a 6 weeks oral treatment with the leukotriene receptor antagonist montelukast improved cognitive functions in aged rats to a level comparable to healthy young rats. Concerning the underlying mechanisms which provoked these functional improvements, we observed, besides increased levels of neurogenesis, an altered microglial morphology, indicating reduced neuroinflammation in aged rats after montelukast treatment. In the treated aged animals, microglia were characterized by a smaller cell soma and by a reduced size of CD68 immunoreactive particles compared to age-matched controls. Since CD68 is a marker for the phagosomal/lysosomal pathway, this finding points towards an altered phagocytic activity of microglia after montelukast treatment.

To proof this and to examine a potential effect of montelukast on phagocytosis in more detail, we aimed to analyze the ultrastructural morphology of microglia of aged vehicle and montelukast treated rats, with special focus on structures involved in phagocytosis (e.g. phagosomes, lysosomes, autophagosomes).

Therefore, aged (20 months) F 344 rats received a 6 weeks daily treatment with 10 mg kg-1 montelukast or vehicle solution, and transmission electron microscopic (TEM) analysis of the hippocampus of these animals was performed. The hippocampi were extracted from glutaraldehyde perfused animals, impregnated with osmium tetroxide and embedded in Araldite-Durcupan. Ultra-thin sections of about 65 nm were cut and transferred on formvar-coated copper grids, contrasted with uranyl acetate and lead citrate and analyzed with a ZEISS EM 910 transmission electron microscope.

To detect effects of montelukast treatment on phagocytosis, we will analyze microglia in the hippocampus of montelukast and vehicle treated aged rats in respect to the number, size and morphology of vesicles involved in the phagocytic pathway (phagosomes, phagolysosomes and lysosomes). In addition, organelles such as mitochondria, Golgi apparatus and endoplasmic reticulum will be examined to address the activation state of these cells.

These findings are essential to gain more insight into the effects of montelukast on microglia, and into the underlying mode of action of the therapeutic effect on cognition in aged individuals.

In a next step, we aim to test if montelukast also affects phagocytic functions in dementias such as AD, paving the way to future clinical translation for the treatment of dementias.

Presynaptic calcium channel *α2δ* subunits are key organizers of glutamatergic synapses

Clemens L. Schöpf, Stefanie Geisler, Ruslan I. Stanika, Marta Campiglio, Gerald J. Obermair

Division of Physiology, Medical University Innsbruck, Austria

clemens.schoepf@student.i-med.ac.at

Auxiliary α2δ subunits regulate the functional membrane expression of the calcium channel complex and modulate current kinetics. In nerve cells their involvement in channel trafficking has been proposed to regulate synaptic release probability. However, until today the role of α2δ subunits in neurons endogenously expressing three α2δ isoforms (α2δ-1 to -3) is not known. To study their synaptic function we established a cellular triple knockout model by employing α2δ-1 shRNA knockdown in cultured hippocampal neurons derived from α2δ-2/-3 double-knockout mice. Axons from triple knockout neurons displayed varicosities resembling presynaptic boutons. These boutons, however, in contrast to control did not recycle FM dyes in an activity-dependent manner, suggesting a severe failure in presynaptic function. This defect was accompanied by a 58% reduction in somatic calcium channel current densities, when compared to wild type or α2δ-2 expressing neurons ($p<0.01$) in patch-clamp experiments. Furthermore, immunofluorescence analyses identified a strongly reduced clustering of the two major presynaptic calcium channels Cav2.1 and Cav2.2. Most importantly, a concomitant reduction in presynaptic synapsin as well as postsynaptic PSD-95 and AMPA-receptor labeling revealed an essential role of α2δ subunits in synapse formation and the trans-synaptic organization of synapses. Finally, presynaptic calcium channel clustering as well as pre- and postsynaptic differentiation in triple knockout neurons could be rescued by the expression of each individual α2δ isoform. Taken together our study identified α2δ subunits as highly redundant key organizers of glutamatergic synapses. They regulate presynaptic calcium channel clustering, presynaptic vesicle accumulation, and also the trans-synaptic organization of postsynaptic receptors.

Generation of a conditional reticulon1 gene (rtn1) knock-out using CRISPR-Cas9 technology

CRISPR-Cas is a novel genome engineering technique that offers greatly facilitated design and evaluation of targeting constructs. Core elements are single guided RNAs (sgRNAs) that bind to genomic DNA with high specificity through complementary base pairing. RNA guided Cas9 endonuclease exerts double strand breaks in the genomic DNA which, together with homology-directed repair, is used to place specific mutations in the genome. To derive a conditional null mutation in the rtn1 gene of the mouse we have designed sgRNAs that target the rtn1 locus, as well as corresponding loxP oligonucleotides. Before proceeding by co-injecting the targeting constructs into a mouse zygote, we have evaluated the targeting efficiency of each construct with a HEK293 cell based reporter assay. RTN1-A is a member of the reticulon family of membrane-embedded proteins which in mammals have been shown to modulate aspects of neuronal morphology and physiology. In order to investigate the impact of RTN1-A on neuronal architecture and axonal regeneration we will make use of the GFP-M mouse strain that has EGFP expressed in neurons in a mosaic manner and thus allows for detection of single neurons and fibers. We have performed preliminary experiments with novel tissue-clearing methods which, in conjunction with light-sheet microscopy and 3D rendering, allow for the visualization of individual neurons and fiber trajectories in vivo.

Victoria Schwaninger[1], Nadine Ortner[2], Jörg Striessnig[2], Lars Klimaschewski[1], Christine E. Bandtlow[1], Rüdiger Schweigreiter[1]

[1]Medical University of Innsbruck, CCB Biocenter/ Neurobiochemistry, Innsbruck, Austria
[2]University of Innsbruck, CCB Biocenter, Department of Phamacology and Toxicology - Institute of Pharmacy, Innsbruck, Austria

victoria.schwaninger@i-med.ac.at

L-type $Ca2^+$ channels in mouse rod bipolar cells

Hartwig Seitter[1], Stefanie Geisler[2], Gerald Obermair[2], Alexandra Koschak[1]

[1]University of Innsbruck, Institute of Pharmacy, Pharmacology and Toxicology, Innsbruck, Austria
[2]Medical University Innsbruck, Division of Physiology, Innsbruck, Austria

hartwig.seitter@uibk.ac.at

L-type calcium channels (LTCC) are key molecular components controlling many physiological processes. In the mouse and human retina CaV1.4 LTCCs are the most prominently expressed mediating synaptic vesicle release. Many mutations in the CACNA1F gene, which encodes for Cav1.4 LTCC α1 subunits, have been shown to cause X-linked visual disorders in humans including incomplete Congenital Stationary Night Blindness. Importantly, expression of CaV1.4 was reported in photoreceptors as well as bipolar cells of the mouse retina. Bipolar cells initiate the partitioning of visual information into parallel pathways that ultimately lead to the retinal extraction of visual features which get transmitted to higher visual brain regions. Existing models of CaV1.4 perturbation, however, allow mainly to study photoreceptor phenotypes. Effects of CaV1.4 mutations on synaptic transmission from bipolar cells can't be investigated because outer retinal function is already disturbed and bipolar cells exert remodelling strategies. The aim of this project is to gain a better insight into the specific function of the CaV1.4 LTCCs on selected retinal cells' physiology and synaptic output. To this end, we will target rod bipolar cells (RBCs) for genetic manipulation. We utilize a Cre driver mouse strain (Pcp2-cre) that restricts Cav1.4 mutations to RBCs which constitute the most light-sensitive pathway for rod signals via AII amacrine cells to ganglion cells. In initial quantitative RT-PCR experiments we already confirmed that Cav1.4 is likely the only LTCC present in RBCs. The role of Cav1.4 in RBC transmission and retinal function will be investigated using micro-electrode array recordings from ex vivo mouse retinas. In this poster we present the principles of this analysis i.e. how we present visual stimuli to the retina and record the activity of ganglion cells, i.e. the spiking output of the retina. The novelty of the mouse model approach is the exclusion of an involvement of photoreceptors, allowing for investigation of the morphological and physiological impact of cell-specific perturbations and therefore linking CaV1.4 to specific retinal functions. Importantly in advanced stages of retinal degenerative diseases, often in the absence of photoreceptors, stimulation of inner retinal neurons – in particular rod bipolar cells – are feasible approaches in the restoration of vision in blind patients. Thus, deeper understanding of the role of Cav1.4 LTCCs for bipolar cell function and their contribution to human retinal pathophysiology is essential, also in the light of potential future therapeutic approaches.

Histone deacetylase inhibition as a neuroprotective therapeutic approach in a transgenic mouse model of multiple system atrophy

Edith Sturm, Lisa Fellner, Florian Krismer, Werner Poewe, Gregor K. Wenning, Nadia Stefanova

Department of Neurology, Innsbruck Medical University, Innsbruck, Austria

edith.sturm@i-med.ac.at

Within the last years, it has been shown that histone deacetylases (HDACs) have been implicated to play an important role in the pathogenesis of neurodegenerative diseases, including Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS). It has been shown that HDAC inhibition possesses neuroprotective effects on neurons in models of PD (Roy, A. et al., 2012) and ALS (Cudkowicz, M.E. et al., 2009). Multiple system atrophy (MSA) is a fatal neurodegenerative disorder with rash progression and late disease onset. MSA is characterized by oligodendroglial alpha-synuclein inclusions (Papp, M.I., Kahn, J.E., Lantos, P.L. 1989; Govindarajan, N. et al., 2011), selective neuronal loss, gliosis leading to parkinsonism, autonomic failure and cerebellar ataxia in any combination (Wenning, G.K. et al., 2004). There is no cure of MSA, which is why we here try to identify novel therapeutic targets for this disease through HDAC inhibition by sodium phenylbutyrate (NaPB), a non-selective pan-HDAC inhibitor, in a transgenic mouse model of MSA. Transgenic PLP-alpha-Synuclein mice overexpressing human alpha-synuclein in oligodendroglia received daily intraperitoneal injections of either NaPB (200mg/kg) or saline over a period of eight weeks. After NaPB treatment, behavioral tests were performed evaluating the motor disabilities of the mice. Immunohistochemistry and western blotting were applied to identify the effects of NaPB treatment in the substantia nigra pars compacta of the MSA brain.
MSA transgenic mice showed reduced acetylation of the nucleosome core protein H3 when compared to healthy wild type mice. NaPB treatment of MSA mice resulted in increased H3 acetylation up to levels of healthy controls. Furthermore, NaPB treatment in the transgenic MSA mouse model was associated with neuroprotection of nigral dopaminergic neurons and reduced oligodendroglial alpha-synuclein inclusion density.
Our data suggest that the pan-HDAC-inhibitor NaPB has a significant neuroprotective effect on nigral dopaminergic neurons of transgenic MSA mice. Therefore, it is of great importance that epigenetic mechanisms including histone modifications should be investigated further as potential targets in MSA therapy, since this can also be related to familiar diseases.

Poster # 87 neuroscience

Targeting TLR4 for disease modification in multiple system atrophy: experimental evidence

Serena Venezia, Violetta Refolo, Nadia Stefanova

Department of Neurology, Innsbruck Medical University, Innsbruck, Austria

serena.venezia@i-med.ac.at

Objective: To assess the efficacy of toll-like receptor 4(TLR4) agonists to ameliorate the functional and neuropathological phenotype of a transgenic mouse model of multiple system atrophy (MSA).
Background: TLR4 plays a role in the clearance of α-synuclein by activated microglia (Stefanova et al., 2011;Fellner et al., 2013) and may serve as a therapeutic target in α-synucleinopathies. While the classical TLR4 agonist lipopolysaccharide (LPS) triggers phagocytic activity and pro-inflammatory responses, monophosphoryl lipid A (MPLA) is a LPS derivative that exhibits unique immunomodulatory properties by triggering phagocytic activity without significant release of toxic cytokines/chemokines by macrophages.
Methods: Transgenic mice overexpressing α-synuclein in oligodendrocytes under the proteolipid protein promoter (MSA mice) were randomized in 4 groups and received weekly intraperitoneal injections of either MPLA, LPS or vehicle. After a 12-week treatment period motor behavior was assessed by the pole and beam test. Brains and plasma samples were collected for further neuropathological and immunological analysis. Statistical analysis to compare groups was done by parametric or non-parametric one-way ANOVA as appropriate.
Results: We detected motor improvement in the MPLA groups as compared to MSA mice treated with vehicle only. Preliminary data point towards significant preservation of dopaminergic neurons in the substantia nigra pars compacta parallel to reduction of the density of α-synuclein inclusions in MPLA-treated MSA mice. While no changes in the cytokine/chemokine profile were detected in the brains of any of the treatment groups, chronic inflammatory response was found in the plasma only of LPS- but not of MPLA-treated MSA mice. Respectively, the survival of LPS-treated mice was significantly decreased, while no mortality was detected in the MPLA or vehicle groups during the 12 weeks observation period.
Conclusion: The detected motor improvement and nigral neuroprotection in MSA transgenic mice receiving MPLA is the first indication to support our hypothesis that TLR4 agonists may be beneficial in MSA transgenic mice. Importantly, MPLA in contrast to LPS showed non-toxic TLR4 agonist activity making it potentially interesting candidate for the therapy of MSA and related α-synucleinopathies.

Cd accumulation and metallothioneins in terrestrial snails and slugs: genetic protein conservation versus variability of response patterns

other Poster # 88

Martin Dvorak[1], Michael Niederwanger[1], Raimund Schnegg[1], Veronika Pedrini-Martha[1], Herbert Lindner[2], Reinhard Lackner[1], Reinhard Dallinger[1]

[1]Institute of Zoology, University of Innsbruck, Innsbruck, Austria
[2]Biocenter Innsbruck - Division of Clinical Biochemistry, Innsbruck Medical University, Innsbruck, Austria

martin.dvorak@uibk.ac.at

In terrestrial snails, cadmium (Cd) accumulation is linked to the expression of Cd-specific metallothionein (CdMT) genes and their encoded proteins. It was shown that in species of the helicid family, these proteins are highly conserved, detoxifying a large proportion of the Cd taken up by the snails. Yet, it was not known whether these proteins and their metal-specific features were also conserved in other families of Stylommatophora. Specimens of Helix pomatia, Cantareus aspersus (Helicidae) and Arion vulgaris (Arionidae) were exposed to Cd-enriched food for 8 days, after which time the Cd accumulation in midgut gland was analyzed, and metal distribution patterns recorded among fractions of gel permeation chromatography. Arion CdMT was further purified by ion exchange and reversed phase (RP) high performance liquid chromatography (HPLC). Its primary sequence was elucidated by de novo protein sequencing applying Electro Spray Mass Spectrometry. In addition, its cDNA sequence was decoded by a molecular approach using RACE-PCR. Results show up to 90% sequence similarity and completely conserved Cys positions between the novel CdMT isoform from Arion vulgaris and the respective CdMT sequences known from the two helicid species. In spite of this, conspicuous species-specific differences were observed with respect to Cd accumulation patterns during the exposure period. It is concluded that in spite of sharing highly homologous Cd detoxifying MT isoforms, the metabolic handling of the metal may vary in a species-specific manner.

Hydration Properties of Ice Binding Proteins

Michael Schauperl, Maren Podewitz, Roland G. Huber, Klaus R. Liedl

Institute of General, Inorganic and Theoretical Chemistry/Theoretical Chemistry

michael.schauperl@uibk.ac.at

Pure water freezes at -37°C, because the formation of ice is kinetically hindered at higher temperatures. The common known property of water, freezing at 0°C results only from impurities in the water acting as ice nuclei. Ice nuclei are substances catalysing the formation of ice. Experimental studies showed, that the most active class of known ice nuclei are proteins. These proteins are called ice nucleation proteins and catalyse the freezing already at low minus degrees
(0 to -5°C).
This phenomenon is especially important, when water is found in small droplets, as it is in the artificial snow production and the formation of clouds in the atmosphere, therefore having an huge impact on the earth climate.
The mechanism of ice nucleation itself is understood poorly in comparison to its relevance. In our studies we used molecular dynamics simulation to understand the underlying principle of ice nucleation. The focus in this study was to describe the hydration properties of water in the surrounding of the protein. It was possible to show that for ice nucleation the enthalpic interaction between the water and the protein has to be low. In comparison to the entropy of the surrounding water molecules, which has to be medium or high. Based on these results, we came up with a new improved explanation for the ice nucleation process of ice nucleation proteins.

$CD4^+$ T cell reactivity to orexin/hypocretin in patients with narcolepsy type 1

Melanie Ramberger, B. Högl, A. Stefani, T. Mitterling, B. Frauscher, A. Lutterotti, M. Reindl

Clinical Department of Neurology, Medical University of Innsbruck, Austria

melanie.ramberger@i-med.ac.at

Introduction: Narcolepsy type 1 is caused by a selective loss of orexin/hypocretin producing neurons in the lateral hypothalamus. A strong genetic association with HLA DQB1*06:02 is well-known and further genes involved in immune modulation were shown to be associated with narcolepsy. Although these associations strongly suggest an involvement of immune cells, the contribution of T cells in pathogenesis is largely unknown.
Materials and Methods: We investigated the orexin specific T cell reactivity in peripheral blood mononuclear cells (PBMC) of narcolepsy patients by a CFSE-based proliferation assay. We thereby compared the $CD3^+CD4^+$ T cell proliferation in response to stimulation with an orexin peptide library of narcolepsy patients with cataplexy to healthy controls. Orexin specific $CD3^+CD4^+$ T cell reactivity was determined by flow cytometry and cytokine (IFN-γ and GM-CSF) analysis after 11 days in culture.
Results: Fifteen patients (100% DQB1*06:02 positive) and 15 healthy controls (47% DQB1*06:02 positive) have been included. Individuals were considered as responders, if the cell division index of $CD3^+CD4^+$ T cells, and the stimulation index of IFN-γ or GM-CSF secretion exceeded the cut-off 3. Using these inclusion criteria, 4/15 patients (27%) and 0/15 controls (0%) showed orexin specific proliferation (p=0.0995).
Conclusions and Outlook: Although we observed a higher orexin specific $CD4^+$ T cell proliferation in narcolepsy patients compared to healthy controls, these data indicate no significant difference in $CD4^+$ T cell reactivity to orexin between the groups by using autologous PBMC. In the near future, we plan to extend our study to investigate antibody-mediated mechanisms and $CD8^+$ T cell reactivity in patients with narcolepsy type 1.

Prostate Cancer Genomes: Complexity at Multiple Levels

Recent large scale profiling efforts provided new insights into the complex architecture of prostate cancer genomes. These studies showed that the majority of prostate cancers harbor recurrent genomic rearrangements involving androgen receptor regulated genes, suggesting that androgen signaling could potentially contribute to the formation of rearrangements and genomic instability in prostate cancer. In addition, widespread clonal heterogeneity has been documented in primary prostate cancer suggesting a high level of intra-individual genomic diversity. Here I will discuss new insights into the spectrum of genomic alterations in prostate cancer and highlight diagnostic challenges in clonally diverse tumors.

Michael C. Haffner

Department of Pathology, Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University, Baltimore, MD, USA

michael.c.haffner@gmail.com

PhD Programme Coordinators

Bernhard Flucher
e-mail: bernhard.e.flucher@i-med.ac.at
web: www.mcbo.at

Christoph Schwarzer
e-mail: schwarzer.christoph@i-med.ac.at
web: www.neurospin.at

Reinhard Würzner
e-mail: reinhard.wuerzner@i-med.ac.at
web: www.horos.at

Local organisers

ÖH elected PhD Representatives

Friederike Tuller
e-mail: friederike.tuller@i-med.ac.at
programme: SPIN

Selma Tuzlak
e-mail: selma.tuzlak@i-med.ac.at
programme: MCBO

Elisabeth Pfeiffenberger
e-mail: elisabeth.pfeiffenberger@i-med.ac.at
programme: MO

PhD Programme Representatives

Georg Altenberger	georg.altenbacher@i-med.ac.at
Christina Brühwasser	christina.bruehwasser@i-med.ac.at
Marco Grasse	marco.grasse@uibk.ac.at
Christian Heinrichs	christian.heinrichs@i-med.ac.at
Manuel Haschka	manuel.haschka@i-med.ac.at
Enrica Paradiso	enrica.paradiso@i-med.ac.at
Sebastian Peer	sebastian.peer@i-med.ac.at
Angelo Pidroni	angelo.pidroni@i-med.ac.at
Barbara Pinheiro	barbara.pinheiro@i-med.ac.at
Christoph Sonderegger	christoph.sonderegger@i-med.ac.at
Martina Überall	martina.naschberger@i-med.ac.at
Chiara Volani	chiara.volani@i-med.ac.at
Catarina Xavier	catarina.gomes@i-med.ac.at
Teodor Yordanov	teodor.yordanov@i-med.ac.at

SZABO SCANDIC

Tirol Standortagentur

GVG Diagnostics

MERCK

charles river

STAR LAB

GVG Genetic Monitoring

CMBI Center for Molecular Biosciences Innsbruck