MEDIZINISCHE UNIVERSITÄT
INNSBRUCK

PROGRAM

4TH MUI-START SYMPOSIUM

CENTER OF CHEMISTRY & BIOMEDICINE, INNRAIN 80

PROGRAM

14:00 H Welcome Address of Prof. Christine Bandtlow (Vice Rector of Research and International Relations)

TIME	*Oral presentations*	Abstract
14:10 – 14:20	**Dr.med.univ. Ramon Tasan (Institute of Pharmacology)** "Characterization of Neurokinin B-expressing neurons in the amygdala complex"	O 01
14:20 – 14:30	**Dr.med. Elke Griesmaier (Department of Pediatrics II)** "Evaluation of sigma-1 receptor ligands to protect against inflammation-sensitized glutamate-induced neonatal brain injury"	O 02
14:30 – 14:40	**Dr.med.univ. Hannes Neuwirt PhD (Dept. of Internal Medicine IV-Nephrologie and hypertension)** "Complement System and MAPK Signaling in Calcineurin-Inhibitor induced Nephrotoxicity"	O 03
14:40 – 14:50	**OA Dr. Gregor Brössner (Department of Neurology)** "Non-invasive measurement of brain temperature in magnetic resonance imaging"	O 04
14:50 – 15:00	**Dr. Michiel Langeslag (Division of Physiology)** "FABRY pain: Understanding pain in FABRY disease"	O 05
15:00 – 15:10	**Cedric Hubert De Smet PhD (Division of Cell Biology)** "A novel lipid sensor in the endosomal membrane"	O 06

TIME	*Oral presentations*	Abstract
15:10 – 15:20	**Mag. Martin Bodner PhD (Institute of Legal Medicine)** "Helena, the hidden beauty: resolving the most common West Eurasian mtDNA control region haplotype at the highest resolution by massively parallel sequencing "	O 07
15:20 – 15:30	**Dr.rer.nat. Oliver Schmidt (Division of Cell Biology)** "Novel ESCRT Functions revealed by systematic Approaches"	O 08
15:30 - 15:40	**Dr.med.univ. Benno Cardini (Department of Visceral, Transplant and Thoracic Surgery)** "Simvastatin and Tetrahydrobiopterin biosynthesis in the prevention of chronic allograft vasculopathy in a murine aortic transplantation model"	O 09
15:40 - 15:50	**Dr.rer.nat. Sebastian Herzog (Div. of Developmental Immunology)** "Regulation of B cell development and function by long non-coding RNAs"	O 10

Poster Presentations *15:50 h – 16:00 h*	Abstract
Dr.rer.nat Laura Zamarian (Department of Neurology) "Framing effects in multiple sclerosis: How patients may be misled by the way medical information is presented"	P 01
Dr. med. Astrid Grams (Department of Neuroradiology) "Intracranial aneurysm as a hypertensive disease"	P 02
Mag.rer.nat. Martin Puhr PhD (Department of Urology) "Identification and assessment of altered miRNA expression profiles to improve early prostate cancer detection"	P 03
Mag.rer.nat. Mario Gründlinger PhD (Division of Molecular Biology) "Peroxisomal import pathways and their role in *A. fumigatus* virulence and adaptation"	P 04
Dr.rer.nat. Johanna Gostner (Division of Medical Biochemistry) "Formaldehyde metabolism – On the role of formaldehyde in inflammation"	P 05
Lourdes Rocamora Reverte PhD (Division of Developmental Immunology) "Glucocorticoid synthesis in the thymus"	P 06

Mag. Dr. Phil. Luca Fava (Division of Developmental Immunology) "The Caspase-2-PIDDosome restrains the proliferative capacity of cells failing cytokinesis"	P 07
Dr.med.univ. Gabriele von Gleissenthall (Department of Biological Psychiatry) "Tryptophan and kynurenine metabolism in alcohol dependent patients in acute and medium-term withdrawal"	P 08
Mag.rer.nat Ingo Bauer PhD (Division of Molecular Biology) "AN4022–A novel HDAC complex component as basis for antifungal therapy"	P 09
Coffee and Poster discussion ***16:00 h – 16:30 h***	

Oral presentations

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

Poster presentations

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

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

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

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

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

Please send your PowerPoint presentation or slide to the Servicecenter-Forschung (maria.perez@i-med.ac.at) no later than the **27th of October 2015**.

ABSTRACTS

Oral Presentation

O 01: Characterization of Neurokinin B-expressing Neurons in the Amygdala Complex

Ramon O. Tasan[1], Gillard Lach[1], James Wood[1], Stefan Pauly[1]
[1]Institute of Pharmacology, Medical University of Innsbruck, Austria

Background: Anxiety disorders constitute a major burden for the society and are characterized by dys-regulation of anxiety and fear. Among the different brain areas involved in the encoding and modulation of fear memories, the amygdala plays an exceptionally important role. Intrinsic and extrinsic amygdala connections are predominantly mediated by glutamate and GABA but the resulting behavioral response is fundamentally shaped by various neuromodulators, including different neuropeptides. Despite extensive investigations, the role of many of these neuropeptides is still poorly understood. Among the neuropeptide family of tachykinins, substance P is promoting anxiety-related behavior, whereas the role of neurokinin B (NKB) that is abundantly expressed in different amygdala nuclei is not clear yet.
Methods: Here, we combined immunohistochemistry with neuronal tract tracing, *in vitro* electrophysiology, opto- and pharmaco-genetics and behavioral testing to characterize NKB and NKB-expressing neurons in the extended amygdala and investigate their contribution to fear conditioning and extinction.
Results: In the basolateral amygdala (BLA), NKB was confined to calretinin and vasoactive intestinal peptide-containing neurons. In the central amygdala, NKB was additionally detected in CRF and somatostatin neurons. NKB neurons were all GABAergic and in the BLA they received polysynaptic inputs from thalamic and cortical sources. Application of NKB onto amygdala slices resulted in increased frequency of inhibitory postsynaptic currents, which were mediated by NK1 and NK3 receptors. Silencing of NKB neurons in the basolateral amygdala promoted the ability to differentiate between fear-related and unrelated stimuli, while in the CEA inhibition of NKB neurons reduced the expression of conditioned fear. Interestingly, inhibition of all BLA GABAergic neurons in VGAT-Cre mice resulted in increased fear and delayed extinction, indicating a highly specific function of NKB neurons in the BLA.
Conclusion: Together our data indicate that NKB-expressing neurons constitute a specific GABAergic population that increases fear expression and promotes the generalization of fear. Further studies will clarify the exact mechanism and the contribution of NKB itself in these processes.

Oral Presentation

O 02: Evaluation of Sigma-1 Receptor Ligands to protect against Inflammation-sensitized Glutamate-induced neonatal Brain Injury

Martina Urbanek[1], Ruslan Stanika[2], Gerald Obermair[2], Karina Wegleiter[1], Ursula Kiechl-Kohlendorfer [1], Elke Griesmaier[1]

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[2]Division of Physiology, Department of Physiology and Medical Physics, Medical University of Innsbruck, Austria

Background: Preterm brain injury still is a relevant problem. In our studies we focus on therapeutic strategies, which after sufficient for data acquisition in the experimental setting, and could be rapidly transferred to a clinical study. Substances acting as sigma-1 receptor ligands were shown to be protective in adult models of brain injury and are already undergoing clinical trials in adult neurological diseases and might also be considered a promising therapeutic option in neonatal brain injury. We have previously shown that the selective sigma-1 receptor agonist Pre-084 (2-(4-morpholinethyl)1-phenylcyclohexane-carboxylate) protects against N-methyl-D-aspartate mediated excitotoxic newborn brain injury.

The aim of this study was to evaluate the effect of Pre-084 in inflammation-sensitized glutamate-induced brain injury in vivo and in vitro.

Methods: In the *in vivo* part of this study we used an established murine model of inflammation sensitized glutamate-induced excitotoxic brain injury. Pups were pre-sensitized by intraperitoneal injections of interleukin-1β from postnatal day one to five, followed by injection of ibotenic acid into the right brain hemisphere to create white (WM) and grey matter (GM) lesions. Immediately afterwards, animals were randomized into 2 groups and injected intraperitoneal (i.p.) one hour after injury with: i) vehicle or ii) Pre-084 0.1µg/g body weight. Endpoint was set at 24 hours after the insult and brains were processed for lesion size and microglial cell activation by use of isolectin -B4 staining. In the in vitro part of this study we used mouse primary hippocampal neurons and primary microglia. After pre-stimulation of microglia with Pre-084 (10µM and 100µM) the cells were treated with either LPS (100ng/ml), glutamate (300µM) or PBS for 2 hours and after a recovery period of 18 hours the supernatant (microglia -conditioned medium [MCM]) from each well was collected for co-culture experiments with hippocampal neurons. Neuronal cell viability was assessed 2 hours after co-culture with MCM using propidium iodide and calcein-AM.

Results: Single application of Pre-084 reduced lesion size in cortical grey (mean length of lesion size ± 1SD: control 780.00 µm ± 495.35 versus Pre-084 433.33 µm ± 116.51; n = 8-9; $p < 0.05$) and underlying white matter (mean length of lesion size ± 1SD: control 767.50 µm ± 489.07 vs. Pre-084 391.11 µm ± 126.14; n = 8-9; $p < 0.05$). Microglial cell activation at the lesion site was significantly lower in Pre-084 treated animals as compared to controls (mean number of isolectin-B4 positive cells ± 1SD: control 36.40 ± 6.96 versus Pre-084 19.93 ± 11.99; n = 5; $p < 0.05$). First data also show a reduction of neuronal cell death in vitro after application of Pre-084 pre-stimulated microglia-conditioned media to primary hippocampal neurons.

Conclusion: We show further data for the protective effect of the selective sigma-1 receptor agonist Pre-084 against neonatal brain injury. Pre-084 reduces inflammation-sensitized glutamate-mediated brain injury and in inhibits microglia activation. Based on our previous studies, we suggest that sigma-1 receptor agonists show considerable promise of playing a role as therapeutic strategy in neonatal brain.

Oral Presentation

O 03: Complement System and MAPK Signaling in Calcineurin-Inhibitor induced Nephrotoxicity

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[1]Department of Internal Medicine IV, Nephrology and Hypertension, Medical University of Innsbruck, Austria

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

Background: The gap between organ demand and supply for transplantation is getting wider. One way to improve the situation is to prolong allograft survival. One entity that significantly contributes to renal allograft loss is calcineurin inhibitor (CNI) nephrotoxicity (CIN). Various mechanisms are discussed to play a role in CIN pathogenesis, one of which is complement mediated injury.

Our aim was to investigate the impact of CNIs on MAPK signaling, complement regulators and complement activation.

Methods and Results: We have performed experiments utilizing two proximal tubule cell lines of human origin: HK2 and hTERT-RPTCs. CyclosporinA (CyA) and tacrolimus (FK506, 10 µM each) treatment induced phosphorylation of MAPK1/-2 in both cell lines. This was associated with a significant decrease in protein levels of suppressor of cytokine signaling (SOCS)-3. Since we have shown recently SOCS-3 acts as a negative regulator of MAPK1/-2 signaling, one might hypothesize that SOCS-3 down-regulation enhances MAPK activation.

In order to screen for alterations in the complement system, we performed qPCR-arrays assessing 15 different complement factors and –regulators. We found that most complement factors were up-regulated upon treatment, whereas complement inhibitors DAF (CD55), MCP (CD46) and also SOCS-3 were significantly down-regulated. The latter result was confirmed by Western blotting. Next, we wanted to establish a connection between CNI-induced MAPK phosphorylation and complement system. For this purpose, cells were treated with PD184161 and AG126, two selective inhibitors of MEK1/-2 and MAPK1/-2, respectively. Under these conditions MAPKs were not phosphorylated. Moreover, expression of C3 (which is consumed during complement activation), DAF, MCP and SOCS-3 were not altered. Next we overexpressed and knocked down SOCS-3 via transient transfection with an overexpression plasmid (pBIG2i) and siRNA. SOCS-3 overexpression ameliorated CNI effects on MAPK and complement compared to vehicle treated cells, whereas knock-down of SOCS-3 enhanced the regulation (phosphorylation of MAPK and down- regulation of DAF, MCP and C3). Of note, SOCS-3 knock-down alone yielded an equal effect as CNI-treatment. Hence, we believe that a part of CIN may be attributed to dys-regulated complement system activation by the SOCS-3-MAPK1/-2 signaling complex, which induces down regulation of complement regulators DAF/MCP.

Finally, we found a significant up-regulation of TCC (terminal complement complex) after CNI-treatment, as measured by ELISA. Cellular proliferation, as assessed by ^{3}H-thymidine incorporation, was about 20% less in cells treated with CyA and FK506 in normal human serum (NHS, containing complement) compared to heat- inactivated normal human serum (HIS, complement factors denatured by heat). Thus, reduced cell growth could be attributed to complement system induced cell death.

Conclusion: CNI-induced MAPK1/-2 phosphorylation is modulated by SOCS-3 and causes down-regulation of complement inhibitors yielding to complement activation and growth inhibition in human proximal tubule cells. This might be one additional pathomechanism of CIN development.

Oral Presentation

O 04: Non-invasive Measurement of Brain Temperature in Magnetic Resonance Imaging

Gregor Broessner[1], Florian Frank[1], Peter Lackner[1], Ronny Beer[1], Raimund Helbok[1], Bettina Pfausler[1], Erich Schmutzhard[1], Jürgen Finsterbusch[2], Michael Verius[3]

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

on temperature distribution in the healthy and injured brain. With use of MRS we are able to measure brain temperature non-invasively in healthy volunteers and patients with different type of brain injury.

Oral Presentation

O 05: FABRY Pain: understanding Pain in FABRY Disease

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Background: Fabry disease is a life-limiting genetic metabolic disorder caused by a deficiency of lysosomal alpha-galactosidase A activity. A recent screening study in Austria discovered Fabry associated mutations in 1 out of 4000 births. The mutations lead to progressive accumulation of glycolipids in numerous cell types including neurons. First symptoms include chronic burning pain with attacks of excruciating pain and sensory losses. Enzyme replacement therapy improves the severity of the systemic disease, however is insufficient to treat the persisting pain. Small fibre neuropathy and the accumulation of glycolipids have been linked to pain in Fabry patients.
Methods: Mice deficient of alpha-Galactosidase A ($Gla^{-/0}$) serves as a model of Fabry disease and is used to investigate Fabry related functional changes in nociceptors. Thermal and mechanical nociception and locomotive performance were monitored from juvenile (w6) to adult (w20) stage in age-matched $Gla^{-/0}$ and wildtype mice. Cultured sensory neurons will be used as a model for nociception in electrophysiological recordings that are specifically designed to reveal functional alterations of $Gla^{-/0}$ vs. wildtype nociceptive neurons. Since deficits in temperature sensation are associated with Fabry pain, the heat-sensitivity of cultured nociceptors was examined.
Results: Six weeks old, juvenile $Gla^{-/0}$ mice displayed a mechanical hypersensitivity when tested with calibrated von Frey filaments. The mechanical hypersensitivity gradually attenuated to levels comparable to wildtype mice in a time frame of 4 weeks. Hargreaves experiments showed that the onset of Fabry related changes in nociceptors starts around postnatal week 12, evident from the attenuation of heat perception in the hindpaws of $Gla^{-/0}$ mice. At the same time, adult $Gla^{-/0}$ mice (24 weeks) developed a hyposensitivity to mechanical stimuli. However, the $Gla^{-/0}$ mice do not display any motor deficits monitored by RotaRod or quantitative assessment of footfalls and gait. Cultured nociceptors from $Gla^{-/0}$ mice did not reveal any differences in heat sensitivity in electrophysiological recordings. Furthermore, the action potentials from 8-9 and 20-22 week old $Gla^{-/0}$ mice are indistinguishable from action potentials of age-matched controls. However, when $Gla^{-/0}$ nociceptors from 20 week old mice are challenged by a prolonged depolarization, the frequency of action potential firing is significantly increased. Analysis of current-voltage relations of peak-inward currents and sustained outward currents revealed significant changes in $Gla^{-/0}$ nociceptors.
Conclusion: Juvenile Gla-/0 mice display mechanical hypersensitivity that over time reverts into loss of mechanical nociception. The loss of heat perception in $Gla^{-/0}$ mice contradicts with the unchanged heat-sensitivity of $Gla^{-/0}$ nociceptors in culture, implying a different mechanism for the loss of heat-sensitivity in $Gla^{-/0}$ mice. Further, the increased hyperexcitability of $Gla^{-/0}$ nociceptors from 20 week old mice could be caused by changed ionic conductances observed in these nociceptors. This could be a cause of spontaneous pain attacks observed in Fabry patients, but still needs further investigation.

Oral Presentation

O 06: A novel Lipid Sensor in the endosomal Membrane

Cedric H. De Smet[1], Giorgia Lamberti[1], Caroline Hermann[1], Gudrun Liebscher[1], Johannes Rainer[2], Reinhard Kofler[2], Christer S. Ejsing[3], and Lukas A. Huber[1]

[1]Division of Cell Biology, Biocenter, Medical University of Innsbruck, Austria

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

[3]Department of Biochemistry and Molecular Biology, University of Southern Denmark, Odense, Denmark

Background: The LAMTOR / Ragulator complex consists of 5 proteins (LAMTOR1-5) anchored to the late endosomal / lysosomal membrane, and is known to regulate mTOR and MAPK signaling and endosomal biogenesis. Recently, it was shown to have a key role in mTOR amino acid sensing.

Methods and Results: Using unbiased large-scale expression studies, we have found that LAMTOR regulates lipid metabolism and adipocyte homeostasis. A transcriptomics study revealed a dramatic down regulation of lipid biosynthesis and up regulation of lipid degradation in LAMTOR2 deficient MEFs and macrophages. Consistently, lipidomics showed that the abundance of the storage lipid cholesterol ester was reduced threefold in LAMTOR2 deficient MEFs. In-depth analysis of these cells showed that lipid droplet count was reduced and, most strikingly, adipocyte differentiation was completely disrupted. Subsequently, mice lacking LAMTOR2 specifically in adipose tissue were generated using the Adipoq-cre/loxP system. These mice display severe defects in brown and white adipose tissue, and excessive fat depots in blood and liver, reminiscent of what is observed in obese and diabetic animals. Transcriptomics indicated decreased mitochondrial activity in these tissues, which may lead to reduced lipid degradation. This could explain the observed phenotypes, and indicates that these mice have defects in thermogenesis of brown adipose tissue and browning of white adipose tissue.

Oral Presentation

O 07: Helena, the hidden Beauty: resolving the most common West Eurasian mtDNA Control Region Haplotype at the highest Resolution by Massively Parallel Sequencing

Martin Bodner[1], Christina Strobl[1], Simone Nagl[1], Catarina Xavier[1], Gabriela Huber[1], Irene Cardinali[2], Ornella Semino[3], Anna Olivieri[3], Francesca Gandini[3], Alessandro Achilli[2], Antonio Torroni[3], Alessandra Iuvaro[1,4], Susi Pelotti[4], Davide Pettener[5], Donata Luiselli[5], Walther Parson[1,6]

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[4]Department of Medical and Surgical Sciences, Institute of Legal Medicine, University of Bologna, Italy
[5]Department of Biological, Geological and Environmental Science, Laboratory of Molecular Anthropology, University of Bologna, Italy
[6]Forensic Science Program, The Pennsylvania State University, PA, USA

Background: Mitochondrial (mt)DNA is a vital tool in forensic genetics when nuclear markers fail to give results or maternal relatedness is investigated. The ~1.1 kbp mtDNA control region (CR) displays highly condensed variation and is therefore routinely typed. In this restricted range, some samples share identical haplotypes and thus convey weak evidence in forensic queries and limited phylogenetic information. However, a matching CR does not imply that the linked coding regions are identical or that the mtDNAs belong to the same phylogenetic lineage. This is especially true for the most frequent West Eurasian mtDNA CR haplotype 263G 315.1C 16519C, which occurs at a frequency of 3-4% in many European populations and is observed in numerous clades within haplogroup H ("Helena").

Methods and Results: We investigated the power of massively parallel complete (~16.6 kbp) mtGenome sequencing in a pan-Italian sample displaying the "most common haplotype". About a third of the ~300 individuals has been analyzed so far: an unexpectedly high coding region diversity rendered nearly every haplotype, previously considered identical, unique, raising the power of discrimination from 0 to almost 100%. Representing a screening of some 10,000 Italians, this study uniquely offers the possibility of investigating the forensically most important haplotype in the currently largest sample set available for a single country.

Conclusion: This study demonstrates the benefit of complete mtGenome sequencing for forensic applications to enforce maximum discrimination, more comprehensive heteroplasmy detection, as well as highest phylogenetic resolution. Sequencing of the complete sample set might reveal particular phylogeographic patterns. Thereby, the project will gain significance beyond forensics by shedding light on human migration and population genetic questions.

Oral Presentation

O 08: Novel ESCRT Functions revealed by systematic Approaches

Oliver Schmidt[1]
[1]Division of Cell Biology, Biocenter, Medical University of Innsbruck, Austria

Background: The ESCRT machinery drives the multivesicular body (MVB) pathway in eukaryotic cells, which regulates cell signaling by selectively targeting ubiquitinated cell surface receptors and other membrane proteins for degradation in lysosomes. Many aspects of how ESCRT-dependent membrane proteome remodeling contributes to cellular homeostasis are still unknown.
Methods and Results: Using unbiased qualitative and quantitative screening approaches in yeast, we elucidate novel physiological roles of ESCRT machinery.
We have recently shown that the ESCRT pathway is essential to maintain free amino acid levels, particularly during nutrient limitation (Müller, Schmidt et al., 2015; eLife). Early during starvation membrane protein degradation via MVBs supplies amino acids for the efficient synthesis of vacuolar hydrolases, which is required to boost the catabolic activity of vacuoles. This is essential to enhance intracellular amino acid recycling further and thereby – together with autophagy – maintains protein synthesis and promotes the extensive proteome remodeling processes that allow cells to complete cell division and to enter quiescence. These findings reveal an important mechanism by which ESCRT-dependent membrane proteostasis maintains cell growth and survival during nutrient limitation.
Using high-throughput genetic screening, we demonstrated a previously unknown genetic link between the MVB pathway and several genes regulating cellular sphingolipid metabolism. With the generous support from the MUI-Start program we have been following up on these initial screening results and established an intimate connection between MVB sorting and sphingolipid homeostasis.
Conclusion: Our integrative systematic approaches have proven very valuable in pinpointing cellular processes, which critically involve MVB sorting, and are expected to reveal further, previously unappreciated functions of the ESCRT machinery.

Oral Presentation

O 09: Simvastatin and Tetrahydrobiopterin Biosynthesis in the Prevention of chronic Allograft Vasculopathy in a Murine Aortic Transplantation Model

Benno Cardini[1], Robert Eiter[1], Rupert Oberhuber[1], Katrin Watschinger[2], David Bernhard[3], Ernst.R. Werner[2], Manuel Maglione[1]

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[3]Department of Cardiac Surgery, Medical University of Innsbruck, Austria

Background: Chronic allograft vasculopathy (CAV) is a major hallmark of chronic rejection and it represents a major obstacle to long-term graft survival following solid organ transplantation. Recently 3-Hydroxy-3-Methylglutaryl-Coenzym-A (HMG CoA)-reductase inhibitors (Statins) have gained interest as a promising approach in minimizing CAV. Since the underlying mechanism of action is still unclear, it was aim of this study to investigate if the protective effect of simvastatin treatment is driven by increased de novo biosynthesis of tetrahydrobiopterin (BH4) -a potent antioxidant and essential cofactor of the nitric oxide synthase enzymes- due to enhanced GTP cyclohydrolase I (GTPCH I) mRNA expression in the graft.
Methods: In a heterotopic murine aortic transplantation model, male Balb/c mice were used as donors. Donors were either pre-treated by a single oral administration of simvastatin (5mg/kg b.w) or treated with the vector PEG 2 hours before organ retrieval. Following 24 hours of cold storage, aortic grafts were transplantated in c57bl6 wild types. Following 2 hours or 28 days of reperfusion we perfomed histological as well as immunohistochemical analyses. Additionaly mRNA expression of vWF, eNOS, GTPCH and GCH1 was determined by RTqPCR. Intragraft BH4-levels were determined by HPLC and eNOS monomer/dimer formation were detected by westernblot.
Results: Preliminary results showed a markedly increased expression of vWF in simvastatin-pretreated grafts reperfused for 28 days compared to the respective PEG-treated group. However statistical significance could not be reached. No statistically significant differences could be observed in regard to GTPCH and GCH1 expression, indipendently from timepoint and treatment. Following 28 days of reperfusion western blot revealed a significantly higher eNOS monomer/dimer formation compared to the respective 2h reperfused groups ($p<0.05$ for all). Intragraft BH4 as wells as histological and immunohistological examinations are in progress.
Conclusion: Our preliminary data could not confirm the hypothesis, that a single oral application of simvastatin leads to an increased GTPCH expression and hence to an increased BH4 biosynthesis. However, due to the still pending examinations a final definitive conclusion can not be drawn.

Oral Presentation

O 10: Regulation of B Cell Development and Function by Long Non-coding RNAs

Alina Wagner[1], Pornpimol Charoentong[2], Anne Krogsdam[2], Jörg Hackermüller[3], Andreas Villunger[1], Sebastian Herzog[1]

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[2]Division of Bioinformatics, Biocenter, Medical University of Innsbruck, Austria
[3]Division of Bioinformatics and Transcriptomics, Helmholtz Centre for Environmental Research, Leipzig, Germany

Background: B lymphocytes, a key cell type of the adaptive immune system, are generated in a complex developmental process that is subdivided into several consecutive steps. Progression through the individual stages is tightly controlled by checkpoints, and defects in any of these developmental phases can result in autoimmunity, immunodeficiency or tumorigenesis. The underlying molecular mechanisms that on the one hand establish a functional immune system, but on the other hand – if deregulated - contribute to diseases, are not completely understood.
Traditionally, research has focussed on the role of protein-coding genes in the regulation of lymphocyte development and function. However, recent data indicate that the non-coding part of the human genome is extensively transcribed and contributes significantly to the orchestrating and fine-tuning of transcriptional programs both in health and disease. Among the non-coding RNA species identified so far, the group of long non-coding RNAs (lncRNAs), defined by a length of more than 200 nucleotides, is the least understood.
Methods: In this pilot study, we aimed to get an initial idea about the role of lncRNA in the B lymphocyte compartment, focussing both on previously known and novel lncRNAs. With respect to the latter, we have isolated distinct B cell stages from murine bone marrow (pro-B, pre-BI, pre-BII, immature B) and spleen (mature B cells, unstimulated or stimulated with anti-CD40L) and have characterized the transcriptome by high throughput sequencing. Out of this transcriptome dataset, we have retrieved lncRNAs that are differentially expressed throughout B cell development, and have validated these results by qPCR in a larger set of samples. At the moment, we are establishing a protocol for CRISPR/Cas9 genome editing, which will enable us to generate loss-of-function mutants of selected lncRNA candidates.
Results: Our preliminary data indicate that B cells express a lot of known and novel lncRNAs, but whether they play an important role is unclear. A subset of these lncRNAs is differentially expressed in the distinct developmental stages, suggesting that they may confer a certain function. We have started to perform *in vitro* perturbation experiments, e.g. the abovementioned loss-of-function approach, to characterize a subset of these lncRNA candidates in more detail.
Conclusion: Our pilot study suggests that lncRNAs are extensively expressed throughout B cell development, but their functional relevance is still unknown. Based on our RNAseq analysis, we have been able to select a set of promising candidates that are currently investigated in more detail. We expect that our ongoing efforts will demonstrate that at least a subset of these lncRNAs is not transcriptional noise, but rather plays an important role in lymphocyte development and function.

Poster Presentation

P 01: Framing Effects in Multiple Sclerosis: How Patients may be misled by the Way Medical Information is presented

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Background: Patients with multiple sclerosis (MS) have to face important decisions as concerns their medical treatment. Risk understanding is essential to actively participate in health care and make informed decisions. Recent investigations have found that patients with MS make poorer decisions than healthy controls. Patients also show a lower perception of risk relative to their physicians. To our best knowledge, no study has assessed framing effects in MS so far. It is unknown to which extent a positive/negative frame affects the patients' interpretation of medical information. Typically, in medical care, people show a more favourable attitude towards positively framed treatments than towards negatively framed treatments. In this study, we expected more pronounced framing effects for the patients relative to controls, and that these framing effects are related to poorer executive functions.

Methods: Patients with relapsing-remitting MS (n=26; mean EDSS 1.60/SD 1.33; mean FSS 35.50/SD 13.07; mean age 41.50/SD 11.35 years; mean education 12.42/SD 2.28 years) were compared to healthy age- and education-matched controls (n=66; mean age 39.51/SD 14.52 years; mean education 13.23/SD 2.23 years). Participants underwent an extensive neuropsychological assessment including tests of executive functions, number processing, decision making, and framing effects. In the framing task, participants evaluated the outcome of an unknown medication on a 7-point scale (n=20 items). Medications were described either in positive terms (positive frame) or in negative terms (negative frame).

Results: In the framing task, both groups evaluated the positively framed medications more positively than the negatively framed medications. These framing effects were more pronounced in the patient group than in the control group, $t(90)=2.18$, $p<.05$. Groups also differed from each other in tests of executive functions (attention span, working memory, logical reasoning) and number processing (quantity comparison, ratio processing), t-tests, $ps<.01$, with the patients scoring lower than the controls. No significant group difference was found in response inhibition, mental complex calculation, and decision making, $ps>.1$. Higher framing effects in the patient group correlated with lower performance in tests of response inhibition and complex mental calculation, $ps<.05$. Response inhibition and mental complex calculation explained 29.6% of variance in the framing effects of patients.

Conclusion: Patients with relapsing-remitting MS may be relevantly biased in the interpretation of medical information by the way this information is presented. Their susceptibility to positive and negative formulations is related to impulse control and calculation abilities. Patients with MS prefer an active role in treatment decisions. Therefore, careful attention should be paid to the way information is presented when we communicate with patients with MS.

Poster Presentation

P 02: Intracranial Aneurysm as a Hypertensive Disease

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Background: One main risk factor for the development of intracranial aneurysms and their rupture may be arterial hypertension, from which arterial calcification displays an epiphenomenon. In addition, there is a known correlation between arterial hypertension and the presence of adrenal tumors, but a correlation between ruptured intracranial aneurysms and adrenal tumors has never been investigated.
Methods: So far 10 patients with aneurysmal SAH were included, who agreed in a magnetic resonance imaging (MRI) examination of the upper abdomen, which was performed between six and 12 months after the SAH. The MRI scans were screened for adrenal masses, and calcifying macroangiopathy of the intracranial vessels was quantified on computed tomography (CT) scans, which have been performed in the acute stage after SAH. In addition 20 age and gender matched controls from a historical patient group, who did not suffer from aneurysmal SAH and who did receive a cerebral CT or a MRI of the upper abdomen, were included.
Results: In the present population one of the SAH patients and none of the controls displayed an adrenal tumor. Patients with ruptured intracranial aneurysms displayed a significantly higher amount of supra-aortal arterial calcification than the controls (p=0.03).
Conclusions: In the preliminary data a positive connection between ruptured intracranial aneurysms and arterial calcification could be confirmed. A positive correlation between ruptured intracranial aneurysms and adrenal masses has to be proven with a larger amount of patients. The inclusion rate of patients so far was lower than expected, mostly due to the clinical condition or a missing consent. However, after slightly modifying the inclusion criteria, we are planning to enroll at least 50 more patients within the next year.

Poster Presentation

P 03: Identification and Assessment of altered miRNA Expression Profiles to improve early Prostate Cancer Detection

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Background: In recent years, much effort has been made to identify and study novel biomarkers like prostate cancer antigen 3 (PCA3) and the fusion gene TMPRSS2:ERG to improve PCa detection but to date, only the biomarker prostate specific antigen (PSA) is routinely used by urologists. However, despite its tremendous value in clinical practice, PSA is not an ideal biomarker. Thus, identification of new molecular biomarkers is urgently required in order to improve an early PCa detection and management. In this context, micro-RNAs (miRNAs) and their potential use as diagnostic and- or prognostic biomarkers have become focus of investigation for many malignancies. Therefore, we want to clarify within this project, whether deregulated expression profiles of specific miRNAs can be used to discriminate healthy from organ confined and metastatic PCa patients with the aim to establish a basis for the development of a diagnostic miRNA biomarker panel to improve early PCa detection.
Methods: In a pilot study, 40 representative radical prostatectomy PCa patients were selected and matched for histopathological parameters like age, Gleason-score and tumor stage. Frozen prostate tissues were macro-dissected in benign and malignant samples for each patient. Total RNAs including miRNAs were isolated with Direct-zol™ RNA MiniPrep kit. The quality of isolated RNA from all tissue samples was determined by assessment of the RNA integrity number (RIN) with the Agilent 2100 bioanalyzer system. Altered miRNA expression profiles were assessed on miRCURY LNA™ microRNA arrays (7th generation) including 3100 capture probes for all known human miRNAs. Selected altered miRNAs were confirmed with qRT-PCR.
Results: The microRNA array data sets revealed more than 40 significantly regulated miRNAs in PCa tissue. The expression of the most promising differentially regulated miRNAs was confirmed by qRT-PCR analysis. For this purpose specific miRNA primer and probe sets were spotted on 384 well costume made miRNA qRT-PCR-array plates. This validation process identified 31 miRNAs which are significantly deregulated in a direct comparison between benign and cancer samples concerning absolute expression and fold change in PCa tissue.
Conclusion: The applicant has identified a specific tissue miRNA signature for PCa. Furthermore, these identified miRNAs can be used to discriminate low from high Gleason score (GSC) tumors and to distinguish different tumor stages.

Poster Presentation

P 04: Peroxisomal Import Pathways and their Role in *A. fumigatus'* Virulence and Adaptation

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Background: Intensive care medicine faces an increase of opportunistic fungal infections. Among these, *Aspergillus fumigatus* has become the most common airborne fungal pathogen. Understanding filamentous fungal physiology on the molecular level will offer potential keys for developing new strategies in diagnostics as well as therapeutics. Peroxisomes are highly dynamic and versatile organelles, which harbour unique fungal pathways (e.g. siderophore biosynthesis, glyoxylate cycle). Proteins are posttranscriptionally imported from the cytosol to the peroxisomal lumen, either through their C-terminal peroxisomal target signal 1 (PTS1) or the less common N-terminal PTS2. PTS1 import is dependent on its soluble receptor PexE(5), whereas PTS2 import depends on PexG(7) and, furthermore, on the fungal specific receptor PexT(20). The aim of the study is to determine the biological role of the two peroxisomal import pathways in *A. fumigatus'* virulence and adaptation to nutrient limited conditions, different carbon sources and various stressors.
Methods: The function of peroxisomal import pathways in *A. fumigatus* was studied by deletion and reconstitution of the *pexE* and *pexT* gene followed by phenotyping under different growth conditions. Virulence was tested in a murine model of invasive pulmonary aspergillosis. Strains were used for subcellular localization studies of peroxisomal GFP-fusion proteins. Culture supernatants were analysed by HPLC. Isolated intra- and extracellular siderophores were photometrically quantified.
Results: Abrogation of PTS1 or PTS2 protein import due to loss of the corresponding receptors PexE or PexT impairs fatty acid utilisation (ß-oxidation) and highly reduces extracellular siderophore content, reflected in lower growth rates under iron limitation. The Δ*pexE* strain, which is a biotin auxotroph, shows delayed and reduced conidiation and impaired radial growth at high temperatures. Remarkably, Δ*pexE* is avirulent and Δ*pex20* is significantly attenuated in virulence testing. Moreover, PTS2 proteins are mistargeted to the cytosol in Δ*pex20*, confirming the suggested indispensible role as a co-receptor for PTS2 import. It has already been shown, that the ergosterol and siderophore biosynthesis are metabolically linked via their common precursor mevalonate. Nevertheless, overexpression of the siderophore biosynthetic enzyme SidI, coupled with the mislocalization from peroxisomes to the cytoplasm, depletes the mevalonate pool for ergosterol biosynthesis, resulting in impaired fungal growth and hypersensitivity to azoles. HPLC analysis of culture supernatants revealed that PTS1 import might be required for the biosynthesis of fungal specific secondary metabolites.
Conclusion: Peroxisomal function of PTS1 and PTS2 dependent protein import is required for siderophore biosynthesis, biotin biosynthesis, growth on fatty acids and virulence in a murine model of pulmonary invasive aspergillosis. Subcellular compartmentalization of mevalonate consuming pathways ensures no disturbing impact on each other. PTS1 (pexE) import might also play a role in the synthesis of *A. fumigatus* secondary metabolites. This is the first detailed description of the PTS1 receptor PexE and the PTS2 co-receptor PexT in an opportunistic human fungal pathogen.

Poster Presentation

P 05: Formaldehyde Metabolism – on the Role of Formaldehyde in Inflammation

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Background: Formaldehyde (HCHO), the simplest aldehyde, is an important metabolic intermediate that is present in all kingdoms. In human blood, formaldehyde concentrations range from 10-100 µM. Formaldehyde shows high reactivity towards cellular molecules due to its electrophilicity. It is detoxified either by formation of hydroxymethylglutathione (HMG), which is oxidized by alcohol dehydrogenase 5 (ALDH5) and subsequently hydrolysed to give formate, or it can be directly oxidized to formate via mitochondrial aldehyde dehydrogenase (ALDH2). Formate then enters the one-carbon pool and is consumed in different biochemical pathways.
Recent data shows that cancer cells can release considerable amounts of formaldehyde and this mechanism is hypothesized to contribute to tissue penetration by metastatic cells. Additionally, formaldehyde is a strong reducing agent and may be able to modulate immune responses by generating a reductive milieu. Since now, the role of endogenous formaldehyde formation under inflammatory conditions has not been investigated in detail.
Aim and Methods: In this study, we analysed whether the stimulation of cells with inflammatory molecules affects formaldehyde and formate metabolism. Expression changes of above mentioned genes were assessed in phytohaemagglutinin (PHA)-stimulated versus unstimulated peripheral mononuclear cells (PBMC). In addition, the potential of formaldehyde to modulate the activity of the central immunoregulatory enzyme indoleamine 2,3-dioxygenase (IDO-1) was investigated.
Results: Stimulation of PBMC led to a shift of formaldehyde catabolic routes indicated by an upregulation of ADH5 and downstream thiolase ACAT1, while ALDH2 was down-regulated. Addition of formaldehyde at physiological concentrations to PBMC dose-dependently suppressed IDO-1 activity and this decrease was closely associated with a decrease in metabolic capacity, as estimated by resazurin reduction.
Conclusion: To summarize, inflammatory conditions induce changes in formaldehyde/formate metabolism. Still it remains to be estimated whether overall concentrations of formaldehyde increase or if there is a shift in the catabolic route only. Despite its importance as a carbon source for several biochemical pathways after its oxidation to formate, we postulate a signaling function of formaldehyde by exerting biphasic effects: The reductive capacity of formaldehyde may favor a reductive milieu and thus suppress Th1-type related immunobiochemical pathways on the one hand, while on the other hand its electrophilic properties may contribute to the activation of stress signaling pathways.

Poster Presentation

P 06: Glucocorticoid Synthesis in the Thymus

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Background: Glucocorticoids (GC) are steroid hormones which take part in a feedback mechanism in the immune system shutting down the inflammatory response. There is increasing evidence that GC are key players in T cell selection in the thymus and are therefore important in shaping the peripheral T cell repertoire. GC are not only produced by the adrenal glands but also in the thymus. Controversial is the origin of the *de novo* GC production in the thymus: thymic epithelial cells (TEC) or thymocytes as the major producers of GC. In light of this discrepancy, our research is based on the potential capability of thymocytes to synthesize GC with special focus on the study of the enzymes involved in this process: the GC synthesizing enzymes CYP11A1 and CYP11B1 and the GC-activating enzyme 11βHSD1.
Methods and Results: In contrast to previous publications, we found CYP11A1 and 11βHSD1 mRNA expression in both thymocytes and TEC, but no detectable levels of CYP11B1 were found. In order to address the effect of GC on T-cell development and selection, we performed fetal thymic organ cultures and we found that low concentrations of GC were able to increase the number of CD4+CD8+ thymocytes whereas higher concentrations induced apoptosis. Conversely, CD4-CD8- cells were more resistant to high GC concentrations. In addition, using the OP9DL1 *in vitro* system, we were able to assess the development of early immature thymocytes to mature T cells and confirm that upon GC treatment CD4+CD8+ thymocytes could not progress in their maturation.
Conclusion: These results suggest that GC may modulate T cell development affecting thymocytes in a different way depending on their maturation status. Currently, we are investigating the possible role of thymus-derived GC on shaping the TCR repertoire and therefore their importance to maintain immune fitness.

Poster Presentation

P 07: The Caspase-2-PIDDosome restrains the proliferative Capacity of Cells failing Cytokinesis.

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Background: Caspase-2 has been implicated in several essential cellular processes, most notably in the DNA damage response (DDR) and the maintenance of genome integrity. Strikingly though, murine models devoid of Caspase-2 or of members of its postulated activation platform, the PIDDosome, failed to reveal the phenotypes expected by a defective DDR, highlighting the fact that the physiological function of Caspase-2 is poorly understood.

Methods: Here we searched for a genuine activator of Caspase-2 by using a series of pharmacological and genetic tools to interfere with the faithfulness of cell division. We employed biochemical and genetic methods to assess the contribution of PIDDosome subunits to the activation of Caspase-2. Finally, murine models were employed to ultimately assess the physiological relevance of the PIDDosome.

Results: Cells failing cytokinesis assembled the PIDDosome, activated Caspase-2, ultimately resulting in reduced proliferative capacity.

Conclusion: Taken together, we postulate that the PIDDosome functions limit the proliferative capacity of cells that failed cytokinesis.

Poster Presentation

P 08: Tryptophan and Kynurenine Metabolism in Alcohol Dependent Patients in acute and medium Term Withdrawal

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Background: Chronic alcohol dependence constitutes a major disease burden in modern society. Although current treatments show a considerable success, the relapse rate in chronic alcohol dependent patients remains high. In order to efficiently treat alcohol dependency, it is vital to understand the underlying complex mechanisms of recovery during alcohol withdrawal. Recent research including our own study has suggested that tryptophan and kynurenine metabolism is profoundly disturbed during alcohol withdrawal both by immune-associated and cortisol-related mechanisms. Kynurenine production has been shown to be intensified during the alcohol withdrawal and to correlate with some alcohol-associated neuropsychiatric symptoms such as affective symptoms and sleep disturbances. The catabolism of kynurenine during alcohol withdrawal and in particular the role of its catabolites, which are substantially neuroactive (whether toxic or neuroprotective) are still unclear.
This study aims to investigate the dynamics of tryptophan metabolism and kynurenine catabolism in alcohol dependent patients during acute and medium-term alcohol withdrawal.
Methods and Results: Twenty nine patients (23 male, 6 female) were enrolled until now, four subjects had to be excluded (substance relapse or deliberate dropouts). The mean age was 44 years, the mean alcohol consumption per day 149,56 g. The self-reporting alcohol consumption (Audit - Alcohol Use Disorders Identification Test) showed a mean score of 30 (score 0 - 40 points). Heavy alcohol consumption seems to involve moderate depressive symptoms. We observed moderately depressive symptoms at the beginning of the alcohol withdrawal (Beck depression inventory, BDI = 24). During the ongoing withdrawal of the substance these symptoms attenuated (BDI score 17 at day 14, 9 after 4 weeks).
Conclusion: As a biochemical basis, we hypothesized that serum concentration of tryptophan, kynurenine and its catabolites change substantially. We assume an increase in kynurenine in these patients with depressive symptoms. However, the levels of these hormones will be measured simultaneously in all patients only at the end of the observation period.
New insights in the mechanisms of recovery from alcoholism could lead to an improvement of therapeutic and rehabilitation schemes for this disease.

Poster Presentation

P 09: AN4022 – A novel HDAC complex Component as Basis for antifungal Therapy

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

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