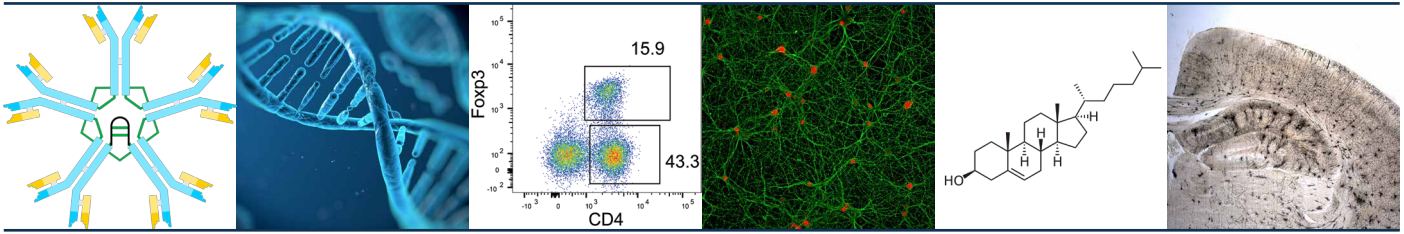
MEDIZINISCHE UNIVERSITÄT
INNSBRUCK

LIFE SCIENCE PhD MEETING INNSBRUCK 2017

universität innsbruck



Life Science PhD Meeting Innsbruck 2017
18th April - 19th April 2017

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The Life Science PhD Meeting provides a platform for students from various scientific backgrounds to share their knowledge, experience and critical thinking. We are proud to present excellent scientific work from numerous fields, which, is only possible due to the sheer variety of scientific interests of the groups, represented in the Meeting. Therefore the organizing committee would like to take the opportunity to thank all the represented research programs:

Medical University of Innsbruck

HOROS (Host Response in Opportunistic Infections)

MCBO (Molecular Cell Biology and Oncology)

SPIN (Signal Processing in Neurons)

Regulation of Gene Expression during Growth, Development and Differentiation

Image-guided Diagnosis and Therapy

Molecular Cell Biology

Musculoskeletal Sciences

Genetics and Genomics

Infectious diseases

Molecular Oncology

Neuroscience

Clinical PhD: Applied Morphology and Regeneration (AMR), Clinical Cancer Research (CCR), Clinical Neurosciences, Intensive Care and Emergency Medicine, Cardiovascular PhD, Clinical Imaging Science

Mission Statement

University of Innsbruck

CMBI (Center for Molecular Biosciences Innsbruck)

Pharmaceutical Sciences

PhD Biology

PhD Chemistry

MCI Technology & Life Sciences

University of Verona

UMIT Health and Life Science University

GSN- LMU

Programme

09:00 - 11:30 M.01.470/490 "Careers in Industry" Workshop (further information: p. 10-11)

11:30 - 12:45 M.01.470/490 *Get-2-gether over lunchtime snacks*

12:50 - 13:15 M.EG.180 Welcome notes

13:15 - 14:00 M.EG.180 Plenary lecture - SPIN, chair: Enrica Paradiso
Catherina Becker, University of Edinburgh, Scottland
Successful regeneration of the spinal cord

14:15 - 15:00 Short talks *parallel sessions*

Chair:	Birgit Lengerer	**Chair:**	Simon Spiegl & Taras Stasyk
14:15 - 14:30 M.EG.180	Muhammad Akram The anti-fungal epoxiconazole inhibits CYP P450 11B1 and CYP P450 11B2: Virtual screening as a tool to find potential endocrine disruptors	**14:15 - 14:30** L.EG.200	Manuel Haschka The dynamics of the apoptosis regulating BCL2 family during extended mitotic arrest
14:30 - 14:45 M.EG.180	Dominik Summer Fusarinine C, a versatile molecule to design novel targeted-imaging probes for oncological applications	**14:30 - 14:45** L.EG.200	Simona Maria Migliano Dynamic of ESCRT-III and Vps4 during reverse membrane budding reactions on endosomes
14:45 - 15:00 M.EG.180	Stefanie Dichtl PCBP2 - A new jigsaw piece in iron dependent host response	**14:45 - 15:00** L.EG.200	Ursula Kahler Linking Conformational States of Thrombin from Experiment and Molecular Dynamics Simulations

15:00 - 17:00 Aula / Foyer Joint poster session #1

Analytical and Diagnostic Methods and Models	Poster # 01-06
Biochemistry and Cell Biology	Poster # 14-27
Developmental Biology and Aging	Poster # 48-53
Genetics and Genomics	Poster # 56-58
Immunity, ID and Clinical Medicine	Poster # 59-69
Pharmacology and Neuroscience	Poster # 81-98

Please be at your poster if your poster-number is:

even	15:00-16:00
odd	16:00-17:00

17:00 - 18:00 Short talks *parallel sessions*

Chair:	Marion Steger & Rita F. Caramalho	**Chair:**	Conor Murphy & Cristina Lemos
17:00 - 17:15 M.EG.180	Oliver Schmidt The multivesicular body pathway and sphingolipid homeostasis	**17:00 - 17:15** L.EG.200	Ahmad Salti The potential of Bmp5/7 on the yield of midbrain dopaminergic neurons during in vitro differentiation of human induced pluripotent and neural stem cells
17:15 - 17:30 M.EG.180	Anna-Maria Dietl Biosynthesis of histidine, pantothenic acid, riboflavin, thiamine and pyridoxine is crucial for virulence of Aspergillus fumigatus	**17:15 - 17:30** L.EG.200	Mehmet Mahsum Kaplan Calcium signals from either L-type calcium channels or ryanodine receptors critically regulate AChR pre-patterning during neuromuscular junction formation
17:30 - 17:45 M.EG.180	Christoph Sonderegger Design and characterisation of improved antifungal peptides	**17:30 - 17:45** L.EG.200	Martina Perwoeg Evaluation and comparison of measured and predicted target registration errors
17:45 - 18:00 M.EG.180	Davide Gerna Bacterial strains on wheat seeds affect hydrogen peroxide production during early seedling growth	**17:45 - 18:00** L.EG.200	Gabriel Bsteh Olfactory threshold predicts short term relapse activity in relapsing-remitting multiple sclerosis

Programme

18:15 - 19:00 Plenary lecture - HOROS, chair: Dorothee Orth-Höller
M.EG.180
Jörg Köhl, University of Lübeck, Germany
Complement controls glucosylceramide accumulationand tissue inflammation in Gaucher disease

19:00 *Cracker, cheese and wine at the posters*
Aula / Foyer

Programme

09:40 - 09:45 Announcements
M.EG.180

09:45 - 10:30 Plenary lecture - MCBO, chair: Teodor Yordanov
M.EG.180
Julia von Blume, MPI of Biochemistry, Martinsried, Germany
Cargo sorting during protein secretion

10:45 - 12:00 Short talks *parallel sessions*

Chair:	Florian Handle & Wilfried Posch	**Chair:**	L. Rocamora-Reverte & Bojana Jakic
10:45 - 11:00 M.EG.180	Anita Erharter A novel human neural stem cells derived 3D in vitro brain model	**10:45 - 11:00** L.EG.200	Carina Miggitsch Human bone marrow adipocytes display distinct functional immune regulatory properties
11:00 - 11:15 M.EG.180	Stefanie Geisler Presynaptic calcium channel α2δ subunits regulate postsynaptic receptor abundance and the wiring of GABAergic synapses	**11:00 - 11:15** L.EG.200	Katia Schoeler Investigating Bim as a functional target of miR-17~92 in tumourigenesis
11:15 - 11:30 M.EG.180	Enrica Paradiso Disinhibitory amygdala microcircuits for aversive learning	**11:15 - 11:30** L.EG.200	Natasa Prokopi Tumor induced immunosuppression: Insights from a spontaneous melanoma mouse model
11:30 - 11:45 M.EG.180	Sinead Rooney The role of microglia/myeloid system in innate anxiety and depression	**11:30 - 11:45** L.EG.200	Victoria Klepsch Beyond CTLA-4 and PD-1: Orphan nuclear receptor NR2F6 as T cell signalling switch and emerging target in cancer immunotherapy
11:45 - 12:00 M.EG.180	Nadine J. Ortner Modulation of Cav1.3 Ca2+ channels in cochlear inner hair cells by RIM-binding proteins	**11:45 - 12:00** L.EG.200	Fabian Schuler Checkpoint kinase 1 (Chk1) is essential for normal development and transformation of B cells in mice

PROGRAMME

12:00 - 13:00 *Lunch break*

13:00 - 15:00 Joint poster session #12
Aula / Foyer

Analytical and Diagnostic Methods and Models	Poster # 07-13
Biochemistry and Cell Biology	Poster # 28-41
Developmental Biology and Aging	Poster # 42-47
Genetics and Genomics	Poster # 54-55
Immunity, ID and Clinical Medicine	Poster # 70-80
Pharmacology and Neuroscience	Poster # 99-115

Please be at your poster if your poster-number is:

even	13:00-14:00
odd	14:00-15:00

15:00 - 17:00 Award ceremony, chair: ÖH PhD Representatives

SPIN best paper award - tba (12 + 3 min)
tba

MCBO best paper award - Michaela Willi(12 + 3 min)
Shin HY , **Willi M.** et al. Hierarchy within the mammary STAT5-driven Wap super-enhancer. 2016. Nature Genetics 48(8):904-11

SPIN Alumni talk - Luca Zangrandi (15 + 3 min)
Synaptic/Extrasynaptic NMDA receptor balance in Alzheimer disease

MCBO Alumni talk - Florian Sparber (15 + 3 min)
Immunity to mucocutaneous fungal infections

CMBI PhD project award

Biooptics image price

Short talks / Poster prices

PROGRAMME

17:15 - 18:00 Plenary lecture - CMBI & ÖH, chair: Bert Hobmayer
M/L.EG.180/200
Arnoud Sonnenberg, Netherlands Cancer Institute, Amsterdam, Netherlands
Molecular basis of cell adhesion to the extracellular matrix

18:00 - 18:05 Closing remarks
M/L.EG.180/200

18:05 *Buffet and grand finale (party)*
Aula / Foyer

Tue 18th
Careers in Industry
09.00 - 13.00

Tyrol-based life science and medtech enterprises introduce their companies as potential future employers and reveal which professional skills are particularly sought after in the various areas of business and industry.

This year, as part of the Life Science PhD Meeting Innsbruck, WTZ West and Standortagentur Tirol's Life Sciences Cluster Tirol warmly invite all those interested to participate in this information and networking event:

09.00 - 09.05 **Welcome address**

09.10 - 10.15 **Company presentations** (5-minute pitches)

10.15 - 11.30 **World Café**
Three rounds of talks, 20 mins. each
Employees from various enterprise sectors will inform students in small groups about their fields of work:

- Research and development/clinical research
- Biomedical engineering
- Regulatory affairs
- Quality assurance
- Analytics
- Other

11.30 - 13.00 **Get-2-gether over lunchtime snacks**

Careers in Industry Workshop

The event is aimed at students/graduates of Tyrolean universities and universities of applied sciences who wish to gain an insight into employment opportunities with both major and smaller players in Tyrol in their fields of study. Smaller discussion sessions enable students/graduates to engage in dialogue with employees from the fields of research and development/clinical research, biomedical engineering, regulatory affairs, quality assurance and analytics.

Who is behind WTZ West?
The objective of the collaboration between the university locations of Salzburg, Innsbruck and Linz is to develop a common culture of knowledge transfer and a mutual tapping of potential for the benefit of not only WTZ West project partners, but also commerce and industry: Generating knowledge together and making such knowledge accessible to the public in the most profitable way for all.

• Short talks - 15min - the speakers have a total of 12min presentation time, followed by 3min discussion

• Poster - the presentation time for each poster is 3min. Your poster will stay at your assigned poster-wall the entire symposium time. Please remove it immediately after the last talk on wednesday. 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

• Posters are sorted by topic:

Analytical and Diagnostic Methods and Models	p. 44-56
Biochemistry and Cell Biology	p. 57-84
Developmental Biology and Aging	p. 85-96
Genetics and Genomics	p. 97-101
Immunity, Infectious Diseases and Clinical Medicine	p. 102-123
Pharmacology and Neuroscience	p. 124-158

The anti-fungal epoxiconazole inhibits CYP P450 11B1 and CYP P450 11B2: Virtual screening as a tool to find potential endocrine disruptors

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M. Patt[2], T. Kaserer[1], V. Temml[1], W. Waratchareeyakul[3], J. Haupenthal[4], R.W. Hartmann[4], A. Odermatt[2], D. Schuster[1]

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PhD programme
Pharmaceutical Sciences

The human beings are exposed to environmental chemicals throughout their life span. Many of the environmental chemicals have the potential to inhibit the production of endocrine hormones. Cortisol synthase (CYP11B1) and aldosterone synthase (CYP11B2) are mitochondrial cytochrome P450 enzymes and are expressed in adrenal cortex of the adrenal gland. Cortisol is a glucocorticoid that monitors the activity of the immune system, blood glucose level, and metabolism of carbohydrates, protein and fats. Any deficiency in CYP11B1 results in the decrease of glucocorticoids, which can lead to the development of Addisonian crisis, cardiovascular collapse, and death. CYP11B2 catalyzes the rate-limiting step in the formation of aldosterone from 18-hydroxycorticosterone. Aldosterone is a mineralocorticoid and regulates the blood pressure by reabsorption of sodium ions at the distal convoluted tubules of the nephrons. CYP11B2 inhibition in healthy individuals can result in life-threatening salt wasting crisis (hyponatraemia), hyperkalemia, hypoaldosteronism, and postural hypotension.
In this study, we report the identification of CYP11B1 and CYP11B2 inhibitors among environmental chemicals using pharmacophore models. The Alanwood pesticides, EU cosmetics, endocrine disruptors, EU food contact, EU food flavoring, international nomenclature of cosmetic ingredients, industrial chemicals, Sigma Aldrich, and drugbank databases were virtually screened by using LigandScout 3.1 (Inte:Ligand GmbH, Vienna, Austria) to find potential anti-target hits. In total, 26 compounds were selected for biological testing. Cell-based assays for CYP11B1 and CYP11B2 inhibition were used for the experimental evaluation of virtual hits. Four of them were dual inhibitors of CYP11B1 and CYP11B2 with IC50s less than 15 μM, representing an overall success rate of 15.4%. The most potent inhibitor was epoxiconazole, an antifungal used in crops production. It inhibited CYP11B1 with an IC50 of 645 nM and CYP11B2 with an IC50 of 113 nM, respectively. The inhibitory effects were further studied in adrenal H295R cells, confirming the potent inhibition of CYP11B1 and CYP11B2 by epoxiconazole. The generated 3D pharmacophore models therefore constitute useful tools for the identification of endocrine disruptors inhibiting CYP11B1 and CYP11B2.

The dynamics of the apoptosis regulating BCL2 family during extended mitotic arrest

Cell death during 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. We could also show that NOXA accumulates in G2 phase and is – similar to MCL1 – degraded during mitotic arrest which lead us to hypothesize that MCL1 and NOXA could be codegraded. To better understand the cell cycle specific regulation of NOXA stability we tested the effect of siRNA mediated knockdown of several E3 ubiquitin ligase complexes described to be involved in the degradation of MCL1, including APC/CCDC20, SCF-FBW7 and MARCH5. While the results of APC/C-CDC20 and SCF-FBW7 depletion were inconclusive MARCH5 knockdown showed a prominent stabilization of NOXA in the later stages of mitotic arrest. This could be confirmed in MARCH5 knock out cells generated with the CRISPR/Cas9 system. These results argue for a strong involvement of MARCH5 in the stability of NOXA and by extension on the progression of extended mitotic arrest.

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PhD programme
Molecular Oncology

Fusarinine C, a versatile molecule to design novel targeted-imaging probes for oncological applications

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PhD programme
Image-guided Diagnosis and Therapy

Over the last decades a broad variety of receptors being overexpressed in cancerous malignancies have been identified as potential targets for therapy and imaging in oncology. As early detection of tumour lesions plays a key role regarding therapy management and is crucial for patients survival, modern imaging techniques e.g. positron emission tomography (PET) and optical imaging as well as the combination of both techniques – hybrid imaging – have gained enormous attention. The general chemical architecture of probes for these imaging approaches consists of a targeting domain - e.g small peptides, affibody or antibody molecules – being conjugated directly or via linker to the signalling moiety. In case of PET the signalling domain is a bifunctional chelator (BFC) forming highly stable complexes towards radionuclides, still offering a free functional group for site specific conjugation of targeting vectors. Fusarinine C (FSC) is a natural occurring cyclic BFC showing excellent complexing properties for radiometals being used for PET like gallium-68 and zirconium-89. Furthermore FSC has three primary amines enabling various capabilities for novel tracer design, being highly advantageous over most commonly used BFC's such as DOTA or NOTA where only one functional group is available to attach target vectors. FSC was used for the concept of multimerization, where up to three targeting peptides (Minigastrin targeting CCK2 receptors) were conjugated in order to increase the tracer concentration at the tumour binding site, leading to enhanced imaging contrast. A proof of principle study was performed to show the feasibility of FSC for dual-modality imaging applying the hybrid imaging concept, combining radiolabelling with a fluorescent dye and 2 targeting vectors on the FSC scaffold. Finally a study was conducted to establish FSC as an alternative to widespread used Deferoxamine (DFO) for affibody molecules radiolabelled with zirconium-89. These studies included chemical conjugation, radiolabelling studies, in vitro characterisation (stability, receptor binding) as well as in vivo imaging studies in mouse tumour models. Here we give a summary on our work with the siderophore based chelator FSC for molecular imaging applications.

Dynamic of ESCRT-III and Vps4 during reverse membrane budding reactions on endosomes

Simona Maria Migliano[1],
M. Alonso Y Adell[1], S. Upadhyayula[2], Y.S. Bykov[3], S. Sprenger[1], M. Pakdel[1,4], G.F. Vogel[1,5], G. Tzu-Yung Jih[2], W. Skillern[2], M. Babst[6], O. Schmidt[1], M.W. Hess[5], J.A.G. Briggs[3,7], T. Kirchhausen[8], D. Teis[1,9]

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PhD programme
Molecular Cell Biology

The ESCRT machinery mediates reverse membrane budding on endosomes, on the nuclear envelop and at the plasma membrane, assisting a multitude of biological processes. ESCRT-III and the hexameric AAA-ATPase Vps4 are the common denominators in all process, but the underlying mechanism and the dynamics of this process are poorly understood. To address these questions, we have generated fluorphore tagged probes for Snf7, Vps24 and Vps4. Using quantitative fluorescence lattice light-sheet microscopy we have analyzed the assembly and the disassembly of endogenous ESCRT-III and Vps4 on endosomes in yeast (Saccharomyces cerevisisae).
Our results show for the first time, that within one second ESCRT-III subunits rapidly polymerize on endosomes and immediately recruit two Vps4 hexamers. Our measurements show a lifetime of 5-45 seconds and also reveal the composition of ESCRT-III assemblies (the number of ESCRT-III molecules in these assemblies), which in consequence recruit several Vps4 hexamers. Instead of remaining in a static or continuously growing polymer, Snf7 and Vps4 hexamers, membrane budding on endosomes strongly requires their continuous exchange. This dynamic exchange between cytosolic and membrane bound ESCRT-III and Vps4 subunits is governed by a stochastic process. Correlative light and electron microscopy (CLEM) underline the recruitment of ESCRT-III and Vps4 subunits to maturing endosomes and multivesicular bodies (MVB). Acute disruption of Vps4 recruitment stalled reverse membrane budding on endosomes as shown by electron tomography. At the end, ESCRT-III and Vps4 are released in a catastrophic all-or-none-process.
From these quantitative determinations, we propose and present a parsimonious model of how ESCRT-III and Vps4 work together during reverse membrane budding on endosomes, which in consequence might allow us to understand other ESCRT dependent cellular processes.

PCBP2 - A new jigsaw piece in iron dependent host response

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PhD programme
Host Response in Opportunistic Infections (HOROS)

Intracellular iron chaperons like Poly(rC)-binding proteins (PCBPs) are not very well investigated in mammalians until now. PCBPs are involved in intracellular iron shuttling. The major isoforms PCBP1 and 2 bind iron with low micromolar affinity and deliver it to proteins and enzymes but are maybe also involved in RNA binding and translational control of gene expression. Thereby, PCBP1 or 2 may affect the expression of the iron transporters DMT1 or ferroportin and the iron storage protein ferritin. Based on lots of open questions regarding the role of PCBPs in macrophages, we investigate the effect of PCBP2 knockout on iron metabolism at base-line and in Salmonella typhimurium (S.tm.) infection.
We developed CRISPR-Cas9 PCBP2 knockout murine J774 macrophages to study the regulation of PCBP2 on iron homeostasis. To find out if PCBP2 is an essential piece in Salmonella dependent host response, we infected the mentioned PCBP2 KO macrophages with the murine pathogenic Salmonella strain Salmonella typhimurium.
A knockout of PCBP2 resulted in a decreased ferroportin level, the major iron exporter, and a higher ferritin expression, which results in an increased intracellular iron content as compared to PCBP2 expressing macrophages. These findings were consistent with an increased uptake of iron and a reduced iron release by PCBP2 depleted cells. To study the consequence of this, in regard to the control of infection with siderophilic intracellular bacteria, we infected wt and PCBP2 depleted macrophages with S.tm.. PCBP2 depletion resulted in an increased intracellular proliferation of Salmonella and reduced infection control. In parallel PCBP2 depletion also impaired anti-microbial immune responses compared to wt macrophages as reflected by an increased expression of IL-6.
Our data demonstrate that PCBP2 is crucial for the control of iron metabolism in macrophages. In Salmonella infection, PCBP2 contributes to host defense by strengthening antimicrobial immune defenses and reducing iron availability for intracellular bacteria. Therefore PCBP2 appears to be an attractive therapeutic target for infections in an era of increasing antimicrobial resistance.

Linking Conformational States of Thrombin from Experiment and Molecular Dynamics Simulations

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PhD programme
Chemistry

Due to its central role in the blood coagulation thrombin is among the most thoroughfully researched enzymes. A wealth of thrombin X-ray structures is deposited in the Protein Data Bank. Their authors have assigned them to different conformational states. The Na+-bound fast form is the most active state, while the anticoagulant slow form lacks the Na+ ion and is less active for most substrates. The structural foundation of thrombin's dyadic nature as procoagulant on the on hand and anticoagulant on the other hand has been subject of controversy.
We used an RMSD metric to compare X-ray structures systematically and find distinguishing and characterizing structural features of the conformational states. The resulting state map facilitates an automatic structure classification, yet fails to yield a clear structural definition for the slow form.
The different activity of fast and slow form was discussed to be connected to their dynamics. Thus we investigated the flexibility of the states with the aid of molecular dynamics (MD) simulations.
The trajectories were clustered and the representatives compared to the states known from X-ray crystallography. With Principal Component Analysis (PCA) the coordinate space is reduced to highlight major differences.
We found that the simulations cover conformations similar to the underlying X-ray structures while augmenting the accessible conformational space.
As metric for local dynamics the alignment-independent dihedral entropies were used. No obvious difference in the flexibility of the conformational forms was observed. However subtle, locally restricted discrepancies became apparent that focalize on the Na+ binding site and support that a purely structural view at thrombin's allostery is insufficient.

The multivesicular body pathway and sphingolipid homeostasis

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Post-Doc

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 pathologie will be discussed.

The potential of Bmp5/7 on the yield of midbrain dopaminergic neurons during in vitro differentiation of human induced pluripotent and neural stem cells

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Post-Doc

Pluripotent stem (PS) cells have been extensively studied in vitro owing to their potential in cell replacement therapies and disease modeling. Transplantation of neural progenitor cells derived from human fetal midbrain tissue in open-label clinical trials, has yielded proof of concept for cell replacement therapy in Parkinson's disease, however, the source of the cells still represents a major limitation. Subsequently, numerous preclinical studies have reported that human PS cells have the ability to differentiate into midbrain dopaminergic (mDA) neurons, and demonstrated the beneficial effects of these cells after transplantation in Parkinson's disease animal models. In vitro approaches typically employ a floor-plate-based protocol, in an attempt to mimic the known developmental events occurring in vivo.
Here, we investigate the role of BMP5/7 signaling on the targeted in vitro differentiation of human PS cells. Thus far, data on the role of BMPs in the differentiation of mDA neurons in vitro have been inconclusive. The most efficient protocols for generating DA neurons from stem cells routinely feature SMAD inhibitors and compounds inactivating BMP activity during early stages to induce neural conversion, but also during later stages, together with other morphogens. However, the purity of DA neurons, that are obtained using current methods, lies approximately between 15-30% of the total neurons generated, suggesting that not all signals required for the differentiation of mDA neurons have been discovered. We applied BMP5/7 during defined stages of DA induction, specification and maturation in vitro and observed a 2 to 3-fold enhancement in the generation of tyrosine hydroxylase (TH)-positive neurons. In order to validate differentiated putative DA neurons more comprehensively we performed immunostainings using A9-type specific combinations of cellular markers such as DAT, LMX1A and GIRK2. Quantification revealed a 3 to 4-fold increase in the number of TH/LMX1A-positive population. TH-positive cells are also co-expressed with DAT and GIRK2 confirming their mDA identity. In conclusion, our study shows involvement of BMB5/7 signaling in DA neuronal specification enabling further insight into mid/hindbrain development and optimization of DA neuron generation for cell replacement therapies.

Biosynthesis of histidine, pantothenic acid, riboflavin, thiamine and pyridoxine is crucial for virulence of Aspergillus fumigatus

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PhD programme
Host Response in Opportunistic Infections (HOROS)

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.
To explore the primary metabolism of A. fumigatus as essential virulence determinant, we generated and characterized auxotrophic A. fumigatus mutant strains defective in seven essential biosynthetic pathways that are absent in mammals. Inactivation of histidine, riboflavin, or pantothenic acid biosynthesis resulted in avirulence, while inactivation of thiamine or pyridoxine biosynthesis resulted in attenuated virulence of A. fumigatus in murine pulmonary infection models. In contrast, the loss of biosynthesis of biotin or siroheme, which plays an important role in nitric oxide detoxification and assimilation of both sulfate and nitrate, did not affect pathogenicity of. A. fumigatus in murine infection models.
These results characterize the host niche with respect to nutrient availability and reveal targets for development of novel antifungal therapeutic approaches.

Calcium signals from either L-type calcium channels or ryanodine receptors critically regulate AChR pre-patterning during neuromuscular junction formation

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PhD programme
Molecular Cell Biology and Oncology (MCBO)

Neuromuscular junction development starts with initial expression and clustering of acetylcholine receptors (AChRs) in the center of the muscle fibers—called AChR pre-patterning—by muscle-intrinsic mechanisms at embryonic day 14.5. A recent study reported an essential role of L-type calcium currents (LTCCs) through skeletal muscle dihydropyridine receptor (DHPR) in this process (Chen et al, 2011). Here, we used various calcium channel mutant mouse models to investigate the role of LTCCs in AChR pre-patterning. As expected, absence of both LTCCs and calcium release from sarcoplasmic reticulum (SR) in muscle of dysgenic mice (DHPR, Cacna1s-/-) led to a failure in AChR pre-patterning and unrestricted branching of the motor nerve, whereas lack of SR calcium release in dyspedic mice (ryanodine receptor, RyR-/-) did not perturb AChR pre-patterning. However, when LTCCs are lacking but EC coupling remains intact, in a mouse model expressing non-conducting DHPR (DHPRnc/nc; Dayal et al., 2014), we observed normal AChR pre-patterning and innervation, demonstrating that LTCCs are dispensable for AChR pre-patterning. This could be explained with two alternative hypotheses. Either DHPR physically regulates AChR pre-patterning by an unknown protein-protein interaction; or, calcium regulates AChR pre-patterning independent of its source (DHPR or RyR). To test these hypotheses we crossed RyR-/- and DHPRnc/nc mice to abolish both calcium signals while DHPR would still be physically present. In this double mutant mouse model (DHPRnc/nc;RyR-/-) AChR pre-patterning failed and diaphragm was hyper-innervated by the motor nerve. Thus, a calcium signal, rather than the physical presence of DHPR, is necessary for regulating AChR pre-patterning and either one calcium source by itself is sufficient to induce AChR pre-patterning. At E18.5, AChR clusters remained broadly scattered and motor nerve covered the entire diaphragm in Cacna1s-/- and DHPRnc/nc;RyR-/- mice, showing that failure in AChR pre-patterning during early development causes aberrant innervation and multiple endplates per myofiber at later developmental stages. Interestingly, nerve branches did not stop at the dispersed AChR clusters and synaptic vesicles failed to correctly aggregate in the nerve terminals, suggesting that lack of postsynaptic calcium signals also causes presynaptic defects.

Design and characterisation of improved antifungal peptides

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PhD programme
Gene Expression

Despite the high frequency and mortality rate of fungal infections (~1.5 million deaths/year), only a few therapeutics are available for their treatment and most have limited effectiveness. Therefore, novel antifungal drugs are urgently needed. A promising candidate for the development of novel antifungal strategies is the naturally occurring antifungal protein PAF, secreted by the well-known industrial penicillin producer Penicillium chrysogenum. For a possible future application it is indispensable to investigate the structure-function relationship of PAF in more detail to potentially enhance its toxicity by protein modelling or to generate peptides that target specific fungal structures.
The 55 amino acid-protein PAF consists of 5 antiparallel β-strands connected by four loops. To investigate the role of the four solvent exposed protein loops in the antifungal activity of PAF, distinct amino acids were exchanged in these regions by site-directed mutagenesis. The amino acids were selected for their charge and polarity, to elucidate the importance of cationic/anionic and hydrophilic/hydrophobic features. Most protein variants showed a significant reduction of the antifungal activity. In contrast, a mutation (PAF T8Y/S10K) in loop1 (γ-core motif) resulted in a gain of antifungal toxicity compared to PAF. Interestingly, the short synthetic peptide PAF14 that spans this γ-core motif (KYTGKCTKSKNECK) exhibited antifungal activity on the PAF-sensitive test organism N. crassa, though at a 240-fold higher MIC (19 μM) than the whole protein. By substituting amino acids in accordance to the PAF T8Y/S10K mutant we created the synthetic peptides PAF14T7Y/S9K and PAF14T7K/K8T/S9K/E12K with improved antifungal activity.
This study identified the γ-core as a key protein motif that is toxic on its own and contributes together with the other protein motifs to the full antifungal activity of PAF. The γ-core motif represents an important mutation site for protein improvement. We could show that rational design of the primary sequence of synthetic peptides originating from the PAF γ-core is a perfect tool for the creation of highly active protein/peptide antifungals.

Evaluation and comparison of measured and predicted target registration errors

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PhD programme
Image-guided Diagnosis and Therapy

Navigation is widely used in ENT-surgery to support the surgeon. A crucial part of the whole navigation process is the registration of the patient to the preoperative CT/MRI-images. Points on the patient and in the image (fiducials) are used to find the transformation between them. Errors made by localizing a fiducial in image and patient space (fiducial localization error (FLE)) lead to the fiducial registration error (FRE), the Euclidean distance between the corresponding fiducials after registration. The target registration error (TRE) indicates the surgeon the accuracy of navigation in the interested area. Knowing the TRE before doing surgery leads to better results of the surgery, less complications during / after surgery and faster healing of the patient. Thus a prediction of the TRE is desirable and different prediction methods were developed.
The aim of this investigation was to compare and analyse predictions and measurements, with data from real experiments under realistic conditions.
For the experiments 3 different patients were registered with pair-point matching registration with 3, 5, 7 and 9 fiducials to their CT-images.
The TRE was calculated pointing on 10 targets points in image and patient space. This process was repeated 10 times for each patient.
6 different estimation methods (isotropic and anisotropic) for TRE-prediction were calculated and compared to measurements. Equality and overestimation of the prediction was statistically tested with a two- and one- sided Wilcoxon signed rank test, alpha = 0.05.
Equality or overestimation of the TRE were found for most of the targets for all patients. A 3- or 5-point registration is enough to get good predictions for the TRE. Overall the predicted TREs are a good estimate for the error at a specific point. The most prominent predictor is approximating the measured TRE in over 50 % of the experiments.
All errors that occur during navigation can be measured and calculated. A complete and detailed evaluation of prediction methods for isotropic and anisotropic FLE was done. It could be shown that prediction of TRE is possible and similar to the results of the measurements.

Bacterial strains on wheat seeds affect hydrogen peroxide production during early seedling growth

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Seed germination and seedling growth are key phases of the plant life-cycle. Importantly, seed performance underpins agricultural productivity, and hence food security. There is an increasing number of reports on seed-microbe associations, including bacterial endophytes, but their contribution to seed germination and vigour are less clear. Here, the seed-microbe interaction was investigated in bread wheat (Triticum aestivum L.). Seeds were surface sterilised with a "hot-steam" treatment, which significantly reduced the number of surface microbes, increased the speed of germination and decreased rates of hydrogen peroxide (H2O2) production, in comparison to non-treated seeds. Considering that an oxidative burst leading to extracellular H2O2 production is a typical biotic stress response of plants, it was hypothesised that bacterial infections were associated to elevated levels of H2O2 production by wheat seedlings. Using Sanger sequencing of the 16S rRNA gene, 20 representative bacterial strains isolated from wheat seeds were identified as Gammaproteobacteria, and either belonged to Pseudomonadaceae or Enterobacteriaceae families. Typical for wheat seeds, the community was abundant in the genus Pantoea, which is known to induce systemic acquired resistance in several plant species investigated. Hot-steam-sterilised seeds were re-inoculated with individual strains, and after 48 h, the level of extracellular H2O2 was measured. Interestingly, two unique strains of Pantoea, including Pantoea agglomerans, which is known for its "Jekyll and Hyde" relationship with plants, induced wheat seedlings to either increase or decrease extracellular H2O2 production. We are currently investigating mechanisms of H2O2 production, and if the differential response by the two strains of Pantoea is related to successful endophytic colonisation and if this may explain changes in seedling growth rates. The effect of treating seeds with other endophytic bacterial strains that have proven growth-promoting abilities is also under investigation. Our overall aim is to improve our understanding of plant-bacterial relationships in seeds and seedlings, and how these interactions influence seed germination and seedling vigour.

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PhD programme
Biology

Olfactory threshold predicts short term relapse activity in relapsing-remitting multiple sclerosis

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Transient impairment of olfactory threshold has been suggested to be a marker of inflammatory disease activity in multiple sclerosis.
To investigate the association of olfactory threshold and disease course in a cohort of relapsing-remitting MS (RRMS) and to determine its use as a potential biomarker of disease activity.
In this prospective, three-year observational study, 141 patients with RRMS were included. Olfactory threshold was assessed using the Sniffin' Sticks at baseline and after 12, 24 and 36 months. Results were correlated with age, sex, disease duration, relapses, expanded disability status scale (EDSS) and disease-modifying treatment (DMT). Predictive value of olfactory threshold for subsequent relapse activity was assessed by Kaplan-Meier estimates and proportional hazard ratios.
Olfactory threshold was significantly impaired in patients relapsing within the subsequent 12 months at baseline (5.5 vs. 6.4; $p<0.041$), at Year 1 (4.8 vs. 6.9; $p<0.001$) and at Year 2 (4.2 vs. 7.0; $p<0.001$). Olfactory threshold was neither associated to relapse within the subsequent 12 to 24 months, nor to age, disease duration, depression, smoking, or EDSS. Patients receiving high-effective DMTs (natalizumab, fingolimod, alemtuzumab) had better olfactory thresholds compared to moderate-effective DMTs (interferon beta, glatirameracetate, dimethylfumarate), patients receiving no DMT or switching DMT during the observation period. A threshold score of 4.5 or lower was associated with an increased risk of relapse in the subsequent 12 months at baseline (HR 3.4; $p<0.001$), at Year 1 (HR 2.7; $p=0.016$) and at Year 2 (HR 3.8; $p=0.003$).
Olfactory threshold is a prognostic marker of short term inflammatory disease activity in RRMS. It is a useful and easily obtainable parameter to stratify patients regarding the level of inflammatory activity and possibly treatment response.

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PhD programme
Clinical PhD

A novel human neural stem cells derived 3D in vitro brain model

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PhD programme
Ageing and Regeneration

Three dimensional structures derived from human suspension cell cultures have emerged as potent in vitro models of various organ systems as they display an extraordinary self-organizing capacity to form functional tissues which resemble whole organs. A recently published protocol enables the generation of cerebral organoids which to a certain extent recapitulate neural progenitor cell proliferation and neuronal differentiation processes in vivo.
Human 3D brain organoid models are expected to close the gap between common two-dimensional in vitro human cell culture and 3D animal models, thus enabling sophisticated assays that will give new insights into early human brain development.
Thus far, human brain organoids have been generated from pluripotent cells only and the potential of neural stem cells to form self-organized 3D structures has not been reported. Here, we will investigate whether or not human induced multipotent induced neural stem cells (iNSCs) are able to form functional neural aggregates. iNSCs, together with neural stem cells derived from other sources, such as human fetal cells, and induced pluripotent stem cells will be tested for their aggregation properties in vitro by using different assays. Spheroids will be implemented in a subsequent approach to generate cerebral organoids. Preliminary data demonstrate that organoid formation capability of neural stem/precursor cells is different to that of pluripotent stem cells. In contrast to them, iNSC form less mature aggregates not exhibiting ventricular structures and layer formation. We assume that since iNSCs are more committed than pluripotent stem cells, it is likely that their characteristics differ in a way that prevents successful aggregation and formation of 3D structures. An explanation might represent the lack of a non-ectodermal component in iNSC-derived 3D structures. Thus, we investigate chimeric constructs containing both pluripotent and neural stem cells to properly implement 3D self-organization. Indeed, we observe particularly layered structures of SOX2-positive cells en face to OCT4-positive pluripotent cells. Moreover, numerous ventricular-like structures expressing characteristic markers such as SOX2, PAX6 as well as pVIMENTIN appear in chimeric 3D aggregates. In conclusion, we demonstrate proof-of-principle of a novel neural 3D organoid paradigm employing defined mixtures of pluripotent and multipotent neural stem cells.

Human bone marrow adipocytes display distinct functional immune regulatory properties

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PhD programme
Host Response in Opportunistic Infections (HOROS)

The bone marrow is a specialized primary lymphoid organ of the human immune system where hematopoiesis takes place. Multipotent hematopoietic stem cells differentiate in the bone marrow to myeloid and lymphoid progenitors which later become most important actors during immune responses. With increasing age, a reduction in bone formation is accompanied by the accumulation of bone marrow fat. No information is yet available on whether adipocytes and their interaction with adaptive immune cells change with age in the bone marrow and whether this type of process is influenced by body weight and the amount, as well as characteristics of fat tissue in other locations. However, the functional effect of bone marrow fat upon immune system has not yet been documented. The primary function of fat tissue is to store excess nutrients in form of energy. Fat produces and secrets adipokines, which can have an effect on various processes such as metabolism and immunity. Especially adipokines exhibit either pro-inflammatory or anti-inflammatory characteristics and contribute to insulin resistance. Here we show that bone marrow fat significantly differs from subcutaneous fat concerning specific gene expression profiles including inflammatory response and adipogenesis. We found low expression levels of adipocyte-specific genes peroxisome proliferator-activated receptor gamma (PPARγ), fatty acid binding protein 4 (FABP4) and high expression of genes like interleukine 10 (IL-10), interleukine 7 (IL-7), interleukin 6 (IL-6), as well as interleukin 15 (IL15) in bone marrow fat. We conclude that immune regulatory markers are crucial in bone marrow fat, contributing to our understanding of age-related changes within the immune system. Answering the question whether the functionality of bone marrow fat differs to subcutaneous fat concerning the immune response in the context of aging will may help developing treatments to prevent loss of immune function, supporting healthy ageing.

Presynaptic calcium channel α2δ subunits regulate postsynaptic receptor abundance and the wiring of GABAergic synapses

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PhD programme
Molecular Cell Biology

Auxiliary α2δ subunits modulate membrane trafficking and current properties of voltage-gated calcium channels and have been implicated in synapse formation. By employing a cellular α2δ triple knockout model in cultured hippocampal neurons, we could recently identify α2δ subunits as key regulators of glutamatergic synaptogenesis. In contrast, synapses of the occasionally observed GABAergic neurons seemed unaffected by knockout of all three isoforms and thus the role of α2δ subunits in GABAergic synapse formation remained unclear. Surprisingly, however, homologous expression of α2δ-2 induced a mismatched localization of postsynaptic GABAA-receptors (GABAARs) opposite glutamatergic terminals, which may indicate a physiological role in GABAergic synapses. This puzzling observation could be explained by several theories: firstly, since over-expression of α2δ-2 increases presynaptic calcium channel abundance, postsynaptic GABAAR may be recruited to compensate for excessive excitatory synaptic activity. In this scenario we expect GABAAR abundance at inhibitory GABAergic synapses to remain unchanged. Secondly, presynaptic α2δ-2 may actively participate in the recruitment and/or anchoring of postsynaptic GABAAR. Here, GABAAR density should be increased both in glutamatergic and GABAergic synapses. Thirdly, over-expression of α2δ-2 could induce axonal rewiring by guiding glutamatergic axons to GABAergic postsynaptic locations.
Therefore, in order to study α2δ subunits in GABAergic synapses we established cultures of striatal neurons, which mainly consist of inhibitory medium spiny neurons. Here I present evidence suggesting that presynaptic α2δ subunits regulate the postsynaptic GABAAR abundance in GABAergic synapses in an isoform specific manner. Moreover, our results show that α2δ-2 recruits GABAARs independent of the presynaptic neurotransmitter identity, which points towards an active involvement of α2δ-2 in the anchoring of postsynaptic GABAARs. Finally, preliminary analysis using super resolution STED microscopy suggests that over-expression of α2δ-2 induces the rewiring of glutamatergic axons to GABAergic postsynaptic sites positioned along dendritic shafts. This is in contrast to the normal situation where glutamatergic synapses are generally formed on dendritic spines. These findings are particularly interesting in light of neuropsychiatric diseases such as autism spectrum disorders, which are associated with axonal wiring defects and have recently been linked to mutations in α2δ subunit genes.

Investigating Bim as a functional target of miR-17~92 in tumourigenesis

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PhD programme
Molecular Oncology

miRNA binding to the 3'UTR of their respective target mRNA modulates the mRNA levels by translational repression and/or degradation. A single miRNA can, according to bioinformatical predictions, regulate hundreds of targets. The challenge at hand is determining functionally relevant targets and discerning direct from indirect targets in the physiological context of primary cells, as well as complex organisms. The miR-17~92 cluster has attracted the fields interest, due to its frequent overexpression in human B-cell lymphomas and its oncogenic potential in mouse lymphoma models.
The miR-17~92 cluster encodes six miRNAs. Based on seed sequence homology, they are categorized into four families: miR-17 (miR-17 and -20), miR-18, miR-19 (miR-19a and -19b) and miR-92a. Target recognition specificity is determined by the distinct sequence of each mature miR-17~92 cluster member. Interestingly, these miRNAs often co-target the same 3'UTRs.
The pro-apoptotic Bcl2 family member Bcl2L11 (Bim) is a top predicted combinatorial and in vitro validated target of the miR-17~92 cluster. Bcl2-like proteins fine-tune the apoptotic threshold, either initiating (Bim, Puma, Bax or Bak) or inhibiting (Bcl2, BclX or Mcl1) apoptosis in all vertebrates.
According to literature Eμ-Myc-driven lymphomas are addicted to miR-17~92 and miR-17~92-mediated Bim repression is of major importance. From a mechanistic point of view, Bim repression potentially permits survival of transforming/ malignant B cells, as well as therapy resistance.
To ultimately test the relevance of the Myc:miR-17~92:Bim axis in lymphoma development and maintenance, we employ a unique in vivo system of conditional mutagenesis wherein we replace the wild type Bim 3'UTR with a miR-17~92 seed-match mutated 3'UTR. Preliminary data confirms the vital importance of the Myc:miR-17~92:Bim axis in vivo: The Bim 3'UTR seed match mutations inhibit miR-17~92:Bim interactions in B cells, hence relieving Bim repression, driving lymphoma cell apoptosis and thereby promoting animal survival. In light of these results, we are exploring whether targeting miR-17~92:mRNA interactions in human B-cell lymphomas, either individually or synergistically with established anti-cancer drugs, holds therapeutic potential.

Disinhibitory amygdala microcircuits for aversive learning

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The basolateral amygdala (BLA) is a cortical-like structure known to be involved in simple forms of emotional learning such as fear conditioning. Local plasticity in the BLA is crucial for fear memory formation and BLA principal neurons (PNs) excitability is under tight control of different subclasses of inhibitory interneurons (INs). However, the contribution of each subtype of INs to sensory processing and fear learning is still poorly understood. Recently, it has been shown that, parvalbumin (PV+) - and somatostatin (SOM+)- expressing INs targeting the perisomatic and dendritic regions of BLA PNs are both inhibited during the presentation of the aversive unconditioned stimulus (US, footshock) in an auditory fear conditioning paradigm. Further, their optogenetic inhibition during the US strengthened the fear memory formation. This suggests a disinhibitory mechanism onto the entire somatodentritic axis of BLA PNs that is likely mediated by a third class of INs. In cortex, VIP+ INs disinhibit pyramidal cells during aversive stimuli by inhibiting PV+ and SOM+ INs.
We therefore hypothesize that VIP+ INs could open a temporal window for associative plasticity in the BLA through PNs disinhibition by inhibiting PV+ and SOM+ INs during the US presentation.
Using a combination of deep brain calcium imaging in freely behaving mice, in vivo optogenetic manipulation during a fear conditioning paradigm and connectivity studies, we show that VIP+ BLA INs are strongly activated by an aversive footshock, that this activation is a mandatory teaching signal for the acquisition of a conditioned fear and that VIP+ INs are embedded in local and long-range circuitry that involve sensory processing and pain perception areas.

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PhD programme
Neuroscience

Tumor induced immunosuppression: Insights from a spontaneous melanoma mouse model

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The metabotropic glutamate receptor 1 (Grm1) is a G-protein coupled receptor that upon binding of its ligand-glutamate- leads to the activation of the MAPK pathway and the PI3K pathway. Therefore, Grm1 ectopic expression in melanocytes confers to them an anti-apoptotic and highly proliferative phenotype, that eventually leads to melanoma formation, in a glutamine-dependent manner. This alteration, that has been detected in 40% of melanoma patient samples, is also present in the tg(Grm1)EPv spontaneous melanoma mouse model. In this mouse model, early tumors are present at the age of 4 months and by the age of 8 months the mice display melanoma lesions with an advanced phenotype.
We have previously shown that immunosuppressive Myeloid Derived Suppressor Cells (MDSC) accumulate in late stage tumors. In addition, we found a strong upregulation of Programmed Death Ligand 1 (PD-L1). By further characterizing the immune composition of the tumors, we saw an increase in CD8+ T cells with tumor growth. Within the total CD8+ population we could observe the expression of inhibitory receptors with tumor progression. This observation was accompanied by the decreased potential of the CD8+ T cells to produce cytokines and granzyme B upon restimulation with anti-CD3/anti-CD28. CD8+ T cells specific for a melanoma-associated antigen (gp100) were found to infiltrate the skin of tumor-free transgenic mice but they gradually decreased with tumor development.
As T cell inhibition in tumors has been described to be a reversible state, we try now to reactivate effector CD8+ T cells by eliminating the potential source of T cell suppression. Based on our previous findings we have started to study the therapeutic potential of MDSC depletion as well as of checkpoint blockade with PD-L1 antibody in this spontaneous melanoma mouse model.

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PhD programme
Molecular Cell Biology and Oncology (MCBO)

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

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Recently, the role of microglia/myeloid cells in stress-induced anxiety or depression has been explored, but has not yet been systematically investigated in 'trait' anxiety and depression. The current project investigates whether a mouse model of innate anxiety and comorbid depressive-like behavior (HAB), in comparison to normal anxiety/depression (NAB), shows neuroinflammatory imbalances and normalization of these pathological events in response to successful treatment. HAB and NAB mice were housed in enriched environment (EE) or standard cage for 4w, and cell counts for microglia/myeloid marker 'Iba1', and co-expression of phagocytic marker 'CD68', were analysed in various brain regions. The enhanced anxiety-like behavior in HABs was correlated with a higher number of Iba1+ cells in the dentate gyrus (DG) of the hippocampus in untreated HABs, compared to NABs. EE decreased and normalized the Iba1+ cell count in the DG of HABs toward that of NABs, in association with successful normalization of anxiety behavior, while CD68+Iba1+ percentage did not change in the DG among all groups. This may indicate that the phagocytic state of microglia in the DG may not contribute to the aberrant anxiety behavior. In addition to the DG, Iba1+ cell populations in the nucleus accumbens and central amygdala were significantly enhanced in HAB compared to NAB, but not altered by EE. Alternatively, Iba1+ cell counts in the cingulate cortex and lateral amygdala did not differ among groups, but untreated HABs showed an increased CD68+Iba1+ percentage in both regions compared to NABs, and EE attenuated this increase. These results suggest that EE-induced reduction of hyperanxiety may involve modulation of microglial phagocytosis in a region-specific manner. Finally, minocycline (inhibitor of 'activated' microglia) rescued the enhanced anxiety-like behavior in HABs. Overall, our results support evidence that disturbances in microglial functioning have an etiological role in innate anxiety/depression-like behavior, and further suggest that microglia could serve as a therapeutic target in some forms of hyperanxiety/depression.

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PhD programme
Pharmacology

Beyond CTLA-4 and PD-1: Orphan nuclear receptor NR2F6 as T cell signalling switch and emerging target in cancer immunotherapy

Modulation of the immune system for the treatment of primary and metastatic tumours in cancer patients has been a goal for many decades. Very recently, blockade of immune checkpoints CTLA-4 and PD-1 has emerged as promising cancer immune therapies. Even though encouraging, there is an unmet medical need, as still only a very limited number of patients respond to and are potentially cured by these therapies. In contrast to cell surface checkpoints, there are cancer therapeutic targets that are located inside the immune cells and are amenable to pharmacological modulation.
Based on our published and unpublished findings that genetically Nr2f6-deficient mice are able to immunologically reject otherwise lethal tumour burdens, we have identified and preclinically validated the orphan nuclear receptor NR2F6 (nuclear receptor subfamily 2, group F, member 6; alias Ear2 and COUP-TFIII) as a bona fide immune checkpoint. We could show that genetic ablation of Nr2f6 significantly improves survival in the murine transgenic TRAMP prostate cancer model. Furthermore, $Nr2f6^{-/-}$ mice spontaneously reject implanted tumours and develop host-protective immunological memory against tumour re-challenge. This is paralleled by increased frequencies of both $CD4^{+}$ and $CD8^{+}$ T cells and higher expression levels of interleukin-2 and interferon-gamma at the tumour site.
This defines NR2F6 as an intracellular and potentially also druggable immune checkpoint, where the presence of NR2F6 limits effector T cell activation within the tumour microenvironment governing the amplitude of anti-cancer immunity, representing a promising avenue for development of alternative immune checkpoint inhibition treatment regimens.

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Post-Doc

Session 1

Modulation of Cav1.3 Ca^{2+} channels in cochlear inner hair cells by RIM-binding proteins

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The Cav1.3 isoform of voltage-gated L-type Ca2+ channels regulates sound-induced tonic neurotransmitter release in cochlear inner hair cells (IHCs). In contrast to other tissues, IHC Cav1.3 channels show very slow Ca2+- (CDI) and voltage-dependent inactivation (VDI). Whereas interaction with CaBP2 can explain the slow CDI, binding of the presynaptic protein RIM2α to the auxiliary β subunit of Cav1.3 reduces VDI – but not to the same extent as observed in IHCs. RIM binding proteins (RBPs) can interact with RIM and with the Cav1.3 C-terminus. We therefore hypothesize that a Cav1.3L-RIM-RBP signaling complex could explain slow Cav1.3 VDI in IHCs.
To determine whether RBP isoforms and RIM proteins are expressed in mouse IHCs, we performed nested PCR. Interaction of RBP2 with the C-terminus of human full-length (Cav1.3L) or short Cav1.3 splice variants (Cav1.3S) was examined using a GST pull-down assay. Functional consequences of complex formation were further investigated by whole-cell patch-clamp recordings (β3, α2δ1, 15 mM Ba2+) in tsA201 cells expressing Cav1.3 channels with and without RIM2α and/or RBP2.
We detected RIM2α, RBP2 and RBP3 transcripts in immature and mature IHCs with nested PCR. GST pull-down revealed binding of RBP2 only to the Cav1.3L C-terminus but not to Cav1.3S. Whole-cell patch-clamp recordings showed a significantly reduced VDI of Cav1.3L upon co-expression of RIM2α and RBP2, while RIM2α or RBP2 alone had less (RIM2α) or no effect (RBP2). This effect was splice variant-specific because when co-expressed with Cav1.3S RBP2 even antagonized the slowing of VDI by RIM2α.
We show that RBP2 and RBP3 are the major isoforms expressed in cochlear IHCs. RBP2 specifically interacts with RIM2α and with the long C-terminus of Cav1.3L. Binding to both is required for slowing VDI to an extent that approaches the slow VDI observed in native IHCs. In the absence of C-terminal binding in Cav1.3S RBP2 can still bind to the channel via RIM2α but prevents its slowing of VDI. Since our previous data have shown the presence of Cav1.3L in IHC ribbon synapses we propose that Cav1.3L-RIM2α-RBP2 complexes contribute to the unique slow VDI relevant for tonic neurotransmitter release in cochlear IHCs.

Checkpoint kinase 1 (Chk1) is essential for normal development and transformation of B cells in mice

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PhD programme
Molecular Oncology

Targeting Checkpoint kinase 1 (Chk1) has been identified as a potential means to kill cancer cells, in particular those lacking functional p53. A number of new generation compounds have been developed and are tested in pre-clinical models and early clinical trials. Yet, as Chk1 is an essential gene, little information is available about its role in normal physiology. Studies using Chk1 haplo-insufficient cells or animals suggested a role as tumor suppressor, in line with its function to halt cell cycle progression and orchestrate DNA repair in response to replication stress or when cells experience exogenous DNA damage. Yet, in cancer cells Chk1 appears to display pro-survival properties, suggesting context dependent activities. Here, we report on the rate limiting role of Chk1 in normal B cell development and MYC-driven transformation as well as lymphoma cell survival, in support of a per se oncogenic function of this kinase. Conditional ablation of Chk1 in the B cell lineage using Mb1-Cre causes a block in B cell development at the pro- to pre-B cell transition. Interestingly, this block cannot be overcome by concomitant overexpression of anti-apoptotic BCL2 that potently blocks pharmacological Chk1 inhibition in vitro, excluding apoptosis as the sole cause of impaired B cell development. Of note, loss of Chk1 precludes the outgrowth of lymphomas in Eμ-MYC transgenic mice, while a reduction in gene-dose delays tumor onset. This effect was associated with an obvious increase in replication stress and hallmarks of DNA damage in premalignant MYC transgenic B cells.
Consistently, human Burkitt lymphoma cell lines as well as Nalm6 pre-B ALL cells are highly susceptible to Chk1 inhibition, indicating that this kinase is critical for tumor cell survival in human malignant disease. Furthermore, Nalm6 cells deficient for BAX and BAK show signs of senescence in response to CHK1 inhibition as a consequence of a nonfunctioning mitochondrial apoptotic machinery. Together, our findings suggest that targeted Chk1 inhibition might be an effective means to treat blood cancers associated with MYC overexpression, yet, given its essential role in normal B cell development, its impact on normal hematopoiesis may severely limit immune competence during therapeutic use.

• The presentation time for each poster is 3min. Your poster will stay at your assigned posterwall the entire symposium time. Please remove it immediately after the last talk on wednesday. 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

• Posters are sorted by topic:

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Poster # 1

Analytical and Diagnostic Methods and Models

CIGuide: an intraoperative hybrid-tracked robotic laser guidance platform

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PhD programme
Image-guided Diagnosis and Therapy

We present a hybrid magnetic tracking based robotic-driven spot-laser guidance system which provides direct visual aid for drilling in cochlear implantology.
The proposed system augments the surgical scene with the help of a visible light laser diode mounted on a robotic needle guidance platform (ISYS). The system uses a novel hybrid approach: magnetic patient tracking (using NDI Aurora) is extended with an iterative visually driven feedback controller that enhances the final accuracy by using direct visual cues provided by reflections of the guidance beam on a dual control plate. In the first step the patient is registered to its preoperative CT images using the Rhinospider technology, which uses a titanium phantom that consists of 4 spherical structures aligned with four 5D Aurora sensors. The device is inserted into the nasopharynx through the nasal cavity before preoperative imaging and stays in place until the end of surgery. This registration technique is user-error free, fully automatic and can provide submillimetric target accuracy. The needle guide with the attached laser diode is positioned in the scene intraoperatively, and its position is tuned with the help of the visual feedback. The feedback uses the reflections of the guidance beam on a tracked and calibrated dual plate. Each plate has a size of 130 x 100 x 3 mm, and are positioned 3 cm away from each other. The upper plate is made from transparent acrylic glass; the lower-plate is made from PEEK, which is sterilizable thermoplastic. An imprinted checkerboard pattern on the lower plate is used to determine the relative positions of the reflections and the alignment of the plates with the help of an external high-resolution camera. Four 5D sensors are mounted on the backside of the lower plate to connect the optical and the magnetic coordinate frames.
In-lab evaluation of our system yielded a target accuracy of 1.2mm (+- 0.5mm), which is good enough for localizing the structures of interest.
Possible applications include all clinical procedures that employ aligning tools. The project is work in progress; we present the test results of a proof-of-concept prototype system in a laboratory setting.

Velocity boundary layer investigation of respiration

Poster # 2

Analytical and Diagnostic Methods and Models

In this contribution the human inhalation process is examined by means of numerical simulation and compared with results. As computational fluid dynamics (CFD) simulation tool lattice Boltzmann (LB) is used, since this method is rather simple applicable on complex geometries. The boundary layer thickness is validated by theoretical background of Blasius.
For flow investigation of the boundary layer (BL) region is crucial, since there the biggest velocity gradients occur. It is also the most difficult region to model within CFD since the mesh quality there has a major impact on accuracy of the overall flow simulation.
The basis for the geometry under investigation is a segmented CT dataset of a human head. Thresholding to air, which has the smallest X-ray density allows to extract a surface geometry of the upper respiratory tract. Due to complexity the nasal floor is cut - where - to allow optical accessibility for the LDA measurements in the future.
Blasius developed an analytical solution of the laminar boundary layer profile and thickness along a flat plate. The main finding is that the BL flow field is self similar with respect to the wall distance made dimensionless with length, viscosity and outer velocity. The smaller the viscosity the smaller the shear stresses and therefore the smaller the boundary thickness will get. Secondly, the BL thickness is inverse proportional to velocity. The comparison between this theoretical background and the complex boundaries of the nasal flow region is in good accordance with the numerical simulation. The boundary layer thickness is about 1mm, whereas the error between simulation and theory is smaller than 0.1 mm.
In the next step LDA measurements validate the boundary layer thickness of the simulation generated by LB.

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PhD programme
Image-guided Diagnosis and Therapy

Poster # 3

Analytical and Diagnostic Methods and Models

Plant Imaging Using Matrix Assisted Laser Desorption Ionization Mass Spectrometry (MALDI-MS)

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PhD programme
Chemistry

The autumnal disappearance of the chlorophylls and the resulting discoloration of leaves is part of one of the most intriguing natural phenomena of life on earth. Recent discoveries of different pathways of the degradation of the green plant pigment have shown remarkable antioxidant properties of some breakdown products. This supports the suggestion that the chlorophyll breakdown pathway may play a more important role in plant tissues than merely removing cytotoxic chlorophyll during plant cell senescence. To further study the chlorophyll breakdown, we investigated the use of MALDI mass spectrometry imaging (MALDI-MSI) as a direct and high throughput analytical technique for molecular profiling and imaging of (senescent) plant tissues.
In general, top regions of a leaf, cuticle and epidermis, which are accessible direct chemical analyses do not contain green plant pigments. Chlorophylls are mainly located in the mesophyll cells while the breakdown products accumulate in the vacuoles of these cells. We developed a direct imaging method for leaf and needle tissues with a simple and rapid sample pre-treatment. We investigated Silver birch (Betula pendula) and conker tree (Aesculus hippocastanum) leaves and the needle of the European Larch tree (Larix decidua)
All MALDI-imaging (and MALDI-PSD) experiments were performed on a Bruker Ultraflex MALDI-TOF instrument. Prior to analysis the leaf and needle tissues were pre-treated with methanol and 2,5 dihydroxybenzoic acid.
The successful development of a simple and direct imaging method suggests the applicability of MALDI-MS for the imaging of broad variety of relevant polyfunctionalized molecules in plant and other biological material.

Microstructure analysis of commercially available medical synthetic bone foams using high resolution micro-computed tomography

Poster # 4

Analytical and Diagnostic Methods and Models

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PhD programme
Image-guided Diagnosis and Therapy

Synthetic bone models were first introduced in late 1980s and used as a bone substitute for biomechanical investigations. Since then, newer generations of these models appear where their structural and mechanical properties are approaching to those found in bone. Even though a variety of models is offered by the manufactures, detailed histomorphometry information is still lacking. The purpose of this study is to perform a microstructural analysis of commercial synthetic open cell foams and find out how similar these models are to real bone in the terms of bone histomorphometry. Compared to existing literature, we will conduct more extensive investigation by encompassing models from two companies (Sawbones and Synbone) and using a larger sample groups.
Eight different, open-cell models were purchased from Sawbones and Synbone companies. Together with human bone samples, we scanned these specimens using high-resolution computed tomography scanner (microCT), and from each of these models 130 cylindrical volumes of interest (diameter 14, height 10 mm) were segmented, binarized and evaluated with 3D histomorphometry tools of Scanco software. Also, due to the structural inhomogeneity within Sawbones samples, additional 2D histomorphometric analysis was performed. In order to validate the data and compare synthetic to bone samples, suitable statistical tests were performed.
2D evaluation results from five Sawbones representing BV/TV, revealed strong structural inhomogeneities in models with higher material densities. Results obtained with statistical analysis of 3D evaluation data shown no significant difference between B5, B7 and B15 Sawbones models compared to vertebral bone for BV/TV parameter; BPL compared to vertebra and femur for Tb.Th parameter; Moon and femur for Tb.Sp and Tb.N parameters; Tube and femur for Conn.D parameter; BPL and vert for DA parameter.
Our investigation revealed strong similarities in certain commercial models compared to human bone. Accordingly, based on the specific research question and in combination with the existing literature, our results could serve as a guideline for those who plan to use these foams in their investigations, whether be it biomechanical or cement augmentation studies.

Poster # 5

Analytical and Diagnostic Methods and Models

[^{68}Ga]FSC-c[RGDfK]$_3$ a multimeric RGD peptide for $\alpha_v\beta_3$ integrin targeting based on a click chemistry

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Gallium-68 labelling of c[RGDfK] via various chelators show high affinity to $\alpha_v\beta_3$ integrin, which is highly expressed during tumour-induced angiogenesis, resulting in high intensity image of positron emission tomography (PET). Fusarinine C (FSC), a three hydroxamates siderophore, reveals the promising bifunctional chelator for ^{68}Ga. Three amino moieties of the backbone can be funtionalised with targeting biomolecules by several conjugation strategies. In recent years "click chemistry", a method for highly selective and high yield reaction under mild conditions, has been intensively used for the synthesis of bioconjugates. Here we introduce the copper (I) catalysed azide-alkyne cycloaddition (CuAAC) click reaction as an alternate conjugation of c[RGDfK] for $\alpha_v\beta_3$ integrin targeting.
The cyclopentapeptide c[RGDfK] was synthesised using solid-phase peptide synthesis (SPPS) and Fmoc strategy. FSC was extracted from A. fumigatus culture and was then complexed with iron (III), FeFSC. An azide moiety was introduced to c[RGDfK] at the side chain NH2 of lysine whereas a terminal alkyne was coupled to FeFSC at three amine functionalities of the backbone. FeFSC-c[RGDfK]$_3$ from CuAAC reaction was demetallated and subsequently allowed radiolabeling of FSC-c[RGDfK]$_3$ with ^{68}Ga. In vitro evaluations including distribution coefficient, protein binding, stability, competition assay, and tumour cell uptake were performed. For in vitro studies, human melanoma $\alpha_v\beta_3$-positive (M21) and $\alpha_v\beta_3$-negative (M21-L) cell lines were used.
CuAAC was successfully applied to the synthesis of FSC-c[RGDfK]$_3$. The radiolabelled compound [^{68}Ga]FSC-c[RGDfK]$_3$ was prepared with high radiochemical yield, (>98%). Distribution coefficient was -2.96±0.2 (n=5) showing a hydrophilic property and protein binding was approximately 10% at 30 and 60 min. [^{68}Ga]FSC-c[RGDfK]$_3$ was stable in PBS (pH7.4) at 37°C for 2 hours. The IC_{50} value was in the low nanomolar range revealing the high affinity to $\alpha_v\beta_3$ integrin. The internalization activity was up to 9.6% of total activity/mg protein for M21 cells as compared to only 0.11% in $\alpha_v\beta_3$ integrin negative M21-L cells showing a receptor specific internalisation of [^{68}Ga]FSC-c[RGDfK]$_3$.
This work demonstrates the possibility of applying click chemistry for preparation of FSC-bioconjugates with comparable properties to other conjugation strategies widening the potential of FSC as scaffold for targeted bioconjugates for molecular imaging applications.

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PhD programme
Image-guided Diagnosis and Therapy

Efficient characterization of local millisecond dynamics: Dihedral entropy from accelerated MD

Poster # 6

Analytical and Diagnostic Methods and Models

We demonstrate a method to capture local dynamics on a time scale three orders of magnitude beyond state-of-the-art simulation approaches. We apply accelerated molecular dynamics for conformational sampling and extract reweighted backbone dihedral distributions. We characterize local dynamics by integration of torsional probabilities, resulting in residue-wise dihedral entropies. We successfully validate our approach for three different protein systems of increasing size: alanine dipeptide, bovine pancreatic trypsin inhibitor (BPTI) and major birch pollen allergen Bet v 1. We demonstrate excellent agreement of flexibility profiles with vast scale computer simulations and experimental dynamics data from NMR. Thus, our method provides efficient access to biologically relevant time scales of local protein dynamics.

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PhD programme
Chemistry

Preclinical evaluation of new radiolabelled minigastrin analogues for diagnostic and therapeutic use in Cholecystokinin-2 receptor expressing tumours

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PhD programme
Image-guided Diagnosis and Therapy

Minigastrin (MG) analogues show high affinity to cholecystokinin-2 receptors (CCK2R) and can therefore be used to target medullary thyroid carcinoma and other CCK2R expressing tumours. To circumvent the limitations of native MG (high kidney retention) and truncated MG analogues (limited stability in vivo) we are investigating new radiolabelled MG analogues in our current FWF project ZFP278440.
Based on MG11 (LAYGWMDF-NH2) four new MG analogues with specific amino acid substitutions on position 6 and 8 were synthesized and derivatized with DOTA at the N-terminus (DOTA-MGS1-4). Using CCK2R-positive AR42J rat pancreatic acinar tumor cells and A431 human epidermoid carcinoma cells transfected with human CCK2R the binding affinity of the peptides and the cell uptake after radiolabelling with In-111 was investigated, including also blocking studies for AR42J cells and uptake studies in mock-transfected A431 cells. The tumour uptake and biodistribution was evaluated in tumour xenografted female athymic BALB/c nude mice and dual modality single photon emission computer tomography (SPECT)/CT images were performed with two analogues showing highest cell uptake.
A half maximal inhibitory concentration (IC50) of 5.5 and 5.8 nM was determined for DOTA-MGS1 and DOTA-MGS4, respectively. DOTA-MGS2 and DOTA-MGS3 showed much lower affinity values (>40 nM). 111In-DOTA-MGS1 (22 ±8 % in AR42J; 25±3% in A431-CCK2R) and 111In-DOTA-MGS4 (9±1 % in AR42J, 20±6 % in A431-CCK2R) showed high receptor-specific cell uptake whereas 111In-DOTA-MGS2 (4.6±0.5 % in AR42J, 3.9±0.5 A431-CCK2R) and 111In-DOTA-MGS3 (0.8± 0.1 % in AR42J, 1.5±0.7 % in A431-CCK2R) showed very much lower uptake values. In the biodistribution studies 111In-DOTA-MGS4 showed the highest tumour uptake (7.1±1.0% ID/g) combined with moderate kidney retention (2.5±0.5% ID/g), whereas 111In-DOTA-MGS1 displayed a value of 2.0±0.4 and 1.1±0.1% ID/g for tumour and kidney, respectively. With both peptide derivatives A431-CCK2R tumour xenografts could be clearly visualised on SPECT/CT images.
From the derivatives studied so far 111In-DOTA-MGS4 seems most promising for diagnostic and therapeutic use. Further studies are ongoing to evaluate the stability of the radioligands against enzymatic degradation. These studies will include stability studies in rat kidney and liver homogenates, as well as biodistribution studies in normal mice analysing the enzymatic stability in vivo.

π-Stacking Interactions of Bicyclic Heteroaromatics

π-stacking plays a central role in medicinal chemistry as an important non-covalent interaction. Due to the importance of this interaction, aromatic rings, able to form this type of interactions, are frequently used as scaffolds in drug design.
In this study density functional theory is used to investigate the stacking interactions of bicyclic aromatic compounds, commonly used in medicinal chemistry. The interaction energies between the bicyclic molecules and a benzene, mimicking a phenylalanine, are investigated on a two-dimensional grid-based approach. This study includes 27 bicyclic aromatic compounds including 6-6-, 6-5- and 5-5-fused aromatic rings frequently used in drug design projects.
We show that stacking interaction can be increased by adding heteroatoms to the bicyclic aromatic system, resulting in a more polarized, electron deficient system. However, the substitution pattern has to be chosen carefully as spatial features strongly influence stacking interaction energies. The presented potential energy surfaces are a helpful tool in drug development to enhance and compare stacking interaction of different scaffolds with each other. Therefore, the obtained results provide valuable information for structure based drug design to enhance ligand affinity and specificity.

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PhD programme
Chemistry

Poster # 9

Analytical and Diagnostic Methods and Models

Quantitative surgery

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PhD programme
Image-guided Diagnosis and Therapy

We present an open source concept, in which by using the navigated surgical stereo microscope with two HD cameras, surface reconstruction of the patient is performed. The obtained point cloud is bound with registration algorithm to a model, segmented from the preoperative images (DICOM), that are registered with the patient, with the use of optical navigation. The resulting point cloud is projected, to the microscope oculars, as an overlay of the patient that defines the certainty of resection borders.
The Leica M500N microscope is calibrated with the optically tracked checkerboard pattern for different zoom and focus levels, that are controlled by can-bus communication. The calibration gives the parameters of cameras (distortion, field of view, rotation and translation) and form an epipolar geometry. Tracking part provides a relation between microscope and checkerboard. With the use of stereo algorithms, characteristic features, that are scale, illumination and occlusion invariant are found in the left and right images, and then matched to define the correct epipolar lines. Homogeneous structures, such is skin, or inhomogeneous ones, that are highly illuminated and provide speculiarities, did not provide enough features to determine a valid disparity map.
Therefore, we exclude the visible light with the bandpass filter and use an NIR source with DLP technology, in order to project an irregular set of points to the surgical scene. This irregularities give enough features, since they form an inhomogeneous structure on the skin.
These features are triangulated with the camera parameters, in order to form a point cloud of the patient surface, with correct scaling, that consist of valid inliers, while outliers are excluded by K-D tree.
We use NDI Optotrak Certus navigation, with which preoperative images are registered with the patient fiducials, and we form a segmented object from the same DICOM series. The microscope surface is bound to a model segmented object, by using the ICP algorithm, that results in the correct alignment of the two point clouds.
By forming the transformation chain and tracking the position of microscope, patient, checkerboard and the DRF, the back projection of the resulting model to the microscope oculars is done.

Poster # 10

Analytical and Diagnostic Methods and Models

Development and validation of work flows for comprehensive metabolomic investigations of human urine, serum and feces with (U)HPLC-QToF-MS and NMR

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PhD programme
Pharmaceutical Sciences

In our body homeostasis is maintained by production of a broad variety of small molecules known as metabolites and any disturbance of this equilibrium leads to changes in quality and quantity of the involved metabolites. In order to achieve a comprehensive insight in our metabolome and to monitor the small differences caused by diseases or lifestyle and nutrition, highly sophisticated analytical techniques like (U)HPLC-QToF-MS or NMR spectroscopy have to be applied. These methods are highly sensitive and hence require sample clean-up without introducing bias. Furthermore reproducible methods for data acquisition are required to facilitate subsequent identification of biomarkers.
In this work we develop sample preparation, data acquisition and processing methods for human urine, serum and fecal samples in order to identify metabolites related to cardiovascular diseases, patients' lifestyle and nutrition.
We developed sample preparation of urine and serum for LC-MS and NMR. For stool samples extraction was optimized and the resulting extract used for both LC-MS and NMR. For all three body fluids we selected columns for chromatographic separation and optimized MS parameters to cover a broad range of metabolites. In NMR we established methods for pH and temperature control as well as pulse programs for acquisition of profile spectra. A suitable system for quality control of measurement and sample preparation was established. Additionally methods for data processing and multivariate statistics were developed which allow identification of metabolites that differentiate between samples from healthy and diseased people. Method development was accomplished by validation of the workflows in which small sample series were investigated.
We were able to develop sample preparation protocols for all three body fluids that can be used for metabolic profiling with both complementary techniques LC-MS and NMR. We have set up a quality control system as well as methods for data acquisition and processing. Terminal validation showed that the developed work flows are sensitive, reproducible and capable to detect the small differences between individuals.
The methods developed and presented in this work serve as a basis for larger scale metabolomic investigations and help to reveal mutual influences between lifestyle/nutrition and vascular diseases.

Poster # 11

Analytical and Diagnostic Methods and Models

Studying the structural diversity of mitochondria phospholipids using a mass spectrometric cardiolipidomics approach

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PhD programme
Genetics and Genomics

Cardiolipins are a mitochondrial class of glycerophospholipid that possess a unique structure, as they carry a hydrophobic tail with four individual acyl side chains and have a polar head group with two glycerol bridged phosphates. Cardiolipins are almost exclusively located in the inner mitochondrial membranes where they make up to 20% of lipid composition. There they are functionally involved in maintaining oxidative phosphorylation by transmembrane protein/ complex stabilisation, buffering the fluctuations of the proton gradient via their unique head group structure, protecting mtDNA, and mediating oxidative damage-related apoptotic signalling events. Their fatty acyl side chain composition is highly regulated by a post biosynthetic remodelling process.
Interestingly, fatty acid profiles vary in a tissue-specific manner to presumably establish maximum functionality of the respiratory chain. Dysfunction of the cardiolipin remodelling pathway by mutations in the Taz gene lead to the development of Barth Syndrome in patients, a severe inherited disease associated with cardiomyopathy, neutropenia, growth delay, muscle weakness and exercise intolerance.
We have recently developed a novel reversed phase high performance liquid chromatography – tandem mass spectrometric methodology, enabling the identification and absolute quantification of up to 140 different cardiolipins within biological samples originating from bacteria, unicellular eukaryotes and whole tissues. In contrast to most previous approaches that target only a few selected cardiolipin subspecies, we are able to analyse a broad spectrum of cardiolipins, including their respective monolyso- and oxidised/ peroxidised counterparts, resulting in a complete cardiolipidome. Furthermore, by mathematical modelling of MS2 spectra generated by data-dependent fragmentation, it was possible to reveal the specific fatty acyl composition of individual cardiolipin species, and to distinguish specific intramolecular side chain and double bond distributions.
Thus, the here presented cardiolipidomics approach grants access to comprehensively characterise this essential mitochondrial signature lipid and to study its role in mitochondrial metabolism and bioenergetics in a broad range of samples.

Poster # 12

Analytical and Diagnostic Methods and Models

3D-Navigation with Radio Frequency Identification (RFIDnav)

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PhD programme
Image-guided Diagnosis and Therapy

Nowadays, to aid surgeons during challenging operations, intraoperative 3D-navigation is employed frequently. This technology employs preoperative radiological patient data in which an actual position of a navigated tool is visualized. Magnetic and optic three-dimensional sensing technology supply poses, i.e. positions and orientations, of tool and patient that are transformed into image space; tool positions are typically visualized as cross-hairs displayed at the calculated image position.
Both optical and magnetic tracking are state-of-the-art in intraoperative navigation. Magnetic sensors can be fabricated very small, but tracking is prone to errors caused by field changes induced by objects with non-negligible magnetic susceptibility: surgical tools are made of steel and are in / near the operating field. Successful use of this technology needs profound knowledge of clinical and surgical processes.
Optical tracking needs uninterrupted line of sight between tracked sensors and the measuring camera. Active and passive technologies IREDs or retro-reflective spheres, may be used for tracking. Basic design considerations imply a minimum size to yield acceptable application accuracy for surgery.
The RFIDnav project explores the potential of RFID-tracking as a potential medical device. The application accuracy of an RFID-scanners operated in the precision mode will be optimized with data fusion approaches. Verification measurements in the lab will show the ultimate application accuracy. This thesis could generate an affordable, precise medical 3D-navigation system with a very low footprint in the operating room. Hence RFIDnav plan will unify the advantages of both tracking technologies: small, exact, robust and precise sensors without cables to allow a precise localization of positions in space.
Using RFID-scanner may be used as a stereoscopic camera with known geometry. This will allow obtaining exact and precise spatial positions by triangulation and will finally be optimized by a Kalman filter, yielding best position estimates and the stochastic uncertainties of the poses.
A prototype in hard- and software will be fully characterized and evaluated against an electromagnetic tracking. Moreover, a clinical demonstrator of a 3D-navigation system will be realized with this technology; this demonstrator will be evaluated in a laboratory setting.

Poster # 13

Analytical and Diagnostic Methods and Models

Fusarinine C, a versatile molecule to design novel targeted-imaging probes for oncological applications

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PhD programme
Image-guided Diagnosis and Therapy

Over the last decades a broad variety of receptors being overexpressed in cancerous malignancies have been identified as potential targets for therapy and imaging in oncology. As early detection of tumour lesions plays a key role regarding therapy management and is crucial for patients survival, modern imaging techniques e.g. positron emission tomography (PET) and optical imaging as well as the combination of both techniques – hybrid imaging – have gained enormous attention. The general chemical architecture of probes for these imaging approaches consists of a targeting domain - e.g small peptides, affibody or antibody molecules – being conjugated directly or via linker to the signalling moiety. In case of PET the signalling domain is a bifunctional chelator (BFC) forming highly stable complexes towards radionuclides, still offering a free functional group for site specific conjugation of targeting vectors. Fusarinine C (FSC) is a natural occurring cyclic BFC showing excellent complexing properties for radiometals being used for PET like gallium-68 and zirconium-89. Furthermore FSC has three primary amines enabling various capabilities for novel tracer design, being highly advantageous over most commonly used BFC's such as DOTA or NOTA where only one functional group is available to attach target vectors. FSC was used for the concept of multimerization, where up to three targeting peptides (Minigastrin targeting CCK2 receptors) were conjugated in order to increase the tracer concentration at the tumour binding site, leading to enhanced imaging contrast. A proof of principle study was performed to show the feasibility of FSC for dual-modality imaging applying the hybrid imaging concept, combining radiolabelling with a fluorescent dye and 2 targeting vectors on the FSC scaffold. Finally a study was conducted to establish FSC as an alternative to widespread used Deferoxamine (DFO) for affibody molecules radiolabelled with zirconium-89. These studies included chemical conjugation, radiolabelling studies, in vitro characterisation (stability, receptor binding) as well as in vivo imaging studies in mouse tumour models. Here we give a summary on our work with the siderophore based chelator FSC for molecular imaging applications.

Phenotypic plasticity of filamentous fungi: chance and challenge

Poster # 14

Biochemistry and Cell Biology

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PhD programme
Biology

Filamentous fungi are biotechnologically important as source of antibiotics like penicillin and cephalosporin or as high yield organic acid producers, especially citric acid, which is widely used as food additive, cosmetic additive or cleaning agent.
These organisms are very sensitive to minor modifications of cultivation parameters like the pH, the medium composition or the oxygen supply and quickly adapt their physiology to environmental changes. The resulting morphological or physiological responses to varying environmental conditions are referred to as phenotypic plasticity. Although screening for novel bioactive metabolites (OSMAC approach) or improving yields of a biotechnologically interesting product make use of this phenomenon, its effects and consequences on experimental issues are still vastly underestimated.
This work emphasizes several phenomena of phenotypic plasticity in filamentous fungi, which have been observed in our laboratory for the past decades. For instance we will demonstrate that the replacement of one nutrient by another, namely glucosamine instead of glucose and/or ammonium, changed the morphological and physiological phenotype in Aspergillus niger and in Penicillium ochrochloron cultures drastically and led to intriguing pigment formation. Using data from a multi-level approach to study organic acid excretion and the dynamics of plasma membrane, energy metabolism and (alternative) respiration, we furthermore highlight the importance of critically reviewing already established methods when transferring them to another phenotype.
Understanding phenotypic plasticity in filamentous fungi but also in microorganisms in general, is in our opinion an additionally important aspect beside reproducibility, repeatability and experimental standardisation in order to compare data from various studies, e.g. new drugs and to achieve progress in the field.

Antitumor active cobalt alkyne complexes derived from acetylsalicylic acid: investigations on the impact of chlorination

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PhD programme
Pharmaceutical Sciences

[(Prop-2-ynyl)-2-acetoxybenzoate]dicobalthexacarbonyl (Co-ASS) is an organometallic derivative of acetylsalicylic acid (ASS). The latter drug acts as irreversible inhibitor of both cyclooxygenase isoenzymes, COX-1 and COX-2. Co-ASS itself displayed high growth-inhibitory potency against certain tumor cell lines. Thereby inhibition of COX-1/2 is assumed as probable mode of action. The isoenzyme COX-2 is mainly related to the development of cancer and abnormal tissue growth, while COX-1 is responsible for homeostatic functions. Hence, a selective inhibition of COX-2 is desirable.

With the aim of generating new compounds, we modified the lead structure Co-ASS by chlorination of the aromatic moiety. The impact of a chlorine substituent regarding cytotoxicity, cellular COX inhibition, potential COX-2 selectivity in particular and cellular uptake compared to the lead structure Co-ASS was evaluated in comprehensive assays.

Cytotoxicity of the compounds was tested in colon cancer (HT-29) and breast cancer (MCF-7, MDA-MB-231) cell lines. The impact of the compounds on the metabolic activity of the respective cell lines was also figured out. Moreover, we analyzed the induction of apoptosis and the inhibition of COX-1/2 was evaluated with human recombinant or ovine isoenzymes. Furthermore, the major COX metabolite prostaglandine E2 (PGE2) was quantified in arachidonic acid treated HT-29 cancer cells. Conclusively cellular uptake studies were conducted by quantifying the cobalt content within the cells upon treatment using atomic absorption spectrometry (AAS).

Chlorination effected a reduction in the extent of COX-1 inhibition compared to Co-ASS. However, the chlorinated derivatives showed reduced COX-2 activity. The reduction was approximately in the same range as by Co-ASS. Interestingly, the compounds did not show any activity in the COX-1/2 negative MCF-7 cell line, while they reduced cell biomass in COX-1/2 positive HT-29 and MDA-MB-231 cells with an IC50 value in the low micromolar range. This selectivity for COX-1/2 expressing cells was confirmed by the other applied cellular assays. The biological profile was complemented by the results of cellular uptake studies.

COX-2 selective cobalt alkyne complexes derived from ASS were characterized as promising antitumor agents. Due to the selective activity in COX-1/2 positive cells, the interaction with the cyclooxygenase cascade can be assumed as potential mode of action.

Do L-type calcium channels share a common mechanism determining voltage sensitivity?

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. An embryonic splicing variant (CaV1.1e) lacking exon 29 located between S3 and S4 of the repeat IV has increased voltage sensitivity and a higher current density, suggesting that exon 29 modulates the mechanism controlling voltage sensitivity. Using structural modeling 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 exon 29 in the adult variant (CaV1.1a) disrupts this interaction, resulting in the poor voltage sensitivity. Given that the residues involved in the D4-R1/R2 interaction are also conserved in L-type calcium channels, we hypothesized that the mechanism governing voltage sensitivity in CaV1.1 is conserved among other CaV family members. To investigate whether this is the case, we analyzed the gating properties of CaV1.2 lacking the exon 33, located between S3 and S4 of the repeat IV, and/or with the D4 charge neutralized. Our results showed that the D4 residue and the exon 33 have only a moderate influence over the voltage sensitivity, indicating that voltage sensitivity is regulated by a different mechanism in the cardiac channel. Interestingly, VSDII contains a similar negative charge in its S3 and a recent publication showed that voltage sensitivity in CaV1.2 is controlled by the VSDII and III. Neutralizing its charge did not affect its gating properties, ruling out the role of D4 interactions in CaV1.2 voltage sensing. CaV1.3 is the L-type calcium channel with the most left-shifted voltage sensitivity of activation. Therefore, we are currently analyzing whether the D4 mutation in IVS3 alters the gating properties of this channel.

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PhD programme
Molecular Cell Biology and Oncology (MCBO)

Functional Analysis of Rbm26 - A Novel Ciliopathy Gene

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Rbm26, as well as its closely related protein Rbm27, are two poorly characterized nuclear proteins, harbouring two RNA recognition motifs (RRM), a PWI domain as well as a CCCH Zn-finger, suggesting a role in RNA metabolism. The Drosophila melanogaster homolog of these two proteins, Swm, is an essential gene and has been described to be essential for entry into S-phase, inhibition of Hedgehog signalling, and required for glycosylation of Notch. In vertebrates, Rbm26 has also been identified in a variety of RNA metabolism-related protein complexes but its precise role is still unclear. We have started to analyse the function of Rbm26 in zebrafish and found that is it required for proper development, proliferation, ciliogenesis and retina formation. Thus, Rbm26 might be novel ciliopathy gene. The main aim of this project is to understand the molecular function of Rbm26/27. To this end, we aim to define the subcellular localization of these proteins and understand whether their expression and localization is cell cycle dependent, determine if they have a role in cellular proliferation and characterize Rbm26/27 protein and RNA interactome. From the results obtained so far, both Rbm26 and Rbm27 show a nuclear pattern by immunofluorescence and Rbm26 expression seems to be cell cycle phase-dependent. To determine its cellular function, Rbm26 shRNA knockdown and Rbm26/27 CRISPR knockout cell lines were generated. Rbm26-depleted cell lines were analysed and these results suggest that Rbm26 might be involved in cell cycle progression and ciliogenesis. Also, Rbm26 depletion leads to a decrease in cellular proliferation. Cell lines for inducible expression of tagged Rbm26 were generated and double affinity purification protocols establish, which will be used to determine Rbm26 associated proteins as well as RNA species.

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PhD programme
Molecular Cell Biology and Oncology (MCBO)

Molecular stress response in *Lumbricus terrestris*

The issue of anthropogenic soil pollution, growing industries and agriculture is very widespread and therefore receiving more attention in the recent years. One of the main soil pollutants is Cadmium (Cd), a highly toxic and carcinogenic heavy metal brought into the environment mainly as a side product in the mining and metal industry. Earthworms are highly important bio indicators and with their soil-dwelling lifestyle well suited to examine the cellular and molecular mechanisms enabling to survive in heavy metal contaminated soils. The earthworm species Lumbricus terrestris has been applied in the present study to characterize the fitness and biomarker response as well as the expression of detoxification-related genes and epigenetic modifications in a long-term experiment (up to seven months) upon environmentally relevant Cd exposure. Our results revealed that the fitness parameters (e.g. weight, reproductive success) and the biomarkers (e.g. DNA damage, Catalase activity, MDA concentration) remained mainly unaffected by Cd, maybe as a result of the activation of detoxification mechanisms like the expression of Metallothionein (MT). MTs are multifunctional, highly conserved proteins playing a major role in metal homeostasis and detoxification processes. A significant increase of MT gene expression was observed upon Cd exposure in a dose and time-dependent manner, whereas the expression of Phytochelatin Synthase (PCS) gene, another protein potentially involved in metal detoxification, was not affected in control and Cd-exposed earthworms. MT expression level indicates the development of acclimation mechanisms. An increase of genome-wide DNA methylation has been found as well, which remained partly modified also after several months of recovery in unpolluted soil. In conclusion we could show that probably due to MT expression no immediate negative influence on individual earthworms could be observed. However epigenetic modifications, which can be persistent and even heritable showing a long-lasting impact across generations, might be found in even moderately polluted environments.

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PhD programme
Biology

Compartmentalization of receptor controlled kinase interactions and conformations

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PhD programme
Chemistry

Cellular membrane receptors sense and convert the vast array of extracellular input signals and relay the information through intricate intracellular signaling circuits. To spatiotemporally control the information flow, it is required to channel the signal through such defined signaling nodes. Hereby, diverse scaffolding proteins take the center stage by interlinking receptors with intracellular effectors. A collection of scaffolds such as A-kinase anchoring proteins (AKAPs), β-arrestin or the kinase suppressor of RAS (KSR) play key roles in redirecting the information flow. We hypothesize that elucidation of either transient protein-protein interactions (PPIs) or whole kinase interaction networks have the potential to reveal conditional signal flow and thus may help to explain pathological implications of distinct kinases. We decided to analyse binary interactions of components of the RAS-RAF-ERK cascade along with the cisphering of macromolecular protein kinase A (PKA) interactions. We developed a unique cell-based reporter system to analyse the mode of interaction of the oncoprotein kinase RAF. Besides determining critical interactions of RAF with the GTPase RAS we managed to track also other kinase conformations upon drug exposure and patient mutation. With this information we are currently investigating ways to perturb these critical protein-protein-interactions either by modulation of the post-translational modification status or by interfering with PPIs. Moreover we developed a strategy to affinity-isolate PKA complexes from different cell types and tissues. We plan to combine affinity isolations with a subtractive phosphoproteomics approach to identify proliferation-relevant PKA interactors and/or substrates which are constituents of cell-specific PKA signalosomes and involved in colon cancer cell proliferation. Interestingly, we already identified known PKA substrates from the RAS-RAF-ERK pathway which we plan to analyse in the context of cancer proliferation.

Assessment of fatty acid oxidation in mouse brain and liver mitochondria

Mitochondrial (mt) respiratory control by substrates and inhibitors represents a key aspect of bioenergetics research. Substrate-uncoupler-inhibitor titration (SUIT) protocols are applied for determining respiratory capacities of selected mt-pathways, with fatty acid oxidation (FAO) becoming a particularly hot topic for mt-fitness. FAO measurement in mt-preparations requires addition of fatty acids and a NADH-linked substrate (malate) to prevent inhibition by accumulating acetyl-CoA. Malate, however, may stimulate respiration above the level of FAO-OXPHOS capacity mainly due to the presence of mt-malic enzyme (mtME). Therefore, conventional SUIT protocols require adjustments for accurate determination of FAO capacity.
In the present study we investigated FAO in liver and brain isolated mitochondria (imt) and tissue homogenate (thom) from C57BL/6 mice. Malate concentration was varied in the range of 0.05 to 10 mM. In liver and brain thom, malate titration stimulated respiration in the presence of ADP. Titration of octanoylcarnitine alone resulted in a modest increase of oxygen consumption in liver imt and thom, suggesting the presence of endogenous substrates. FAO was saturated by malate at 0.1 mM (M.1), whereas mtME required 2 mM. FAO capacity is obtained accurately as the increase of respiration when titrating fatty acid, octanoyl-carnitine, after ADP and M.05.These results illustrate the requirement of strict quality control of SUIT protocols and critical evaluation of metabolic assumptions made for mitochondria studied in different tissues and species.

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PhD programme
Infectious Diseases

Poster # 21

Biochemistry and Cell Biology

In search of novel human lipocalin receptors

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Lipocalins are a broad family of proteins, which transport small hydrophobic molecules such as steroids, bilins, retinoids, and lipids. They share limited regions of sequence homology but a common tertiary structure architecture. These proteins are found in gram-negative bacteria, vertebrates, invertebrates, and in plants. Lipocalins have been associated with many biological processes, among them immune response, inflammation, detoxification, pheromone transport, biological prostaglandin synthesis, retinoid binding, and cancer cell interactions. Because most lipocalins are extracellular proteins their intracellular effects depend on interaction with specific cellular receptors. Although progress in this field has accelerated in recent years the number of lipocalin receptors identified is still limited. Therefore, the major goal of this project is to perform a search for novel lipocalin receptors with a focus on human proteins.
In a first attempt we focused on the identification of receptors for lipocalin allergens. This was performed by two approaches: First, the identification of lipocalin allergen binding peptides by phage display followed by a database search for related membrane protein sequences and second, a ligand-receptor crosslinking strategy coupled to affinity purification followed by HPLC-MS identification of proteins pulled down. Potential candidate receptors will subsequently by expressed in E. coli or insect cells and tested in vitro for lipocalin binding by Isothermal Titration Calorimetry or Surface Plasmon Resonance. In vivo relevance of found interactions will be probed by ligand uptake studies in human cell lines following specific receptor candidate knockdown by siRNA/shRNA methods.

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PhD programme
Gene Expression

Study the function and biological significance of HAT1 in Drosophila melanogaster

Poster # 22

Biochemistry and Cell Biology

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?

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PhD programme
Molecular Cell Biology and Oncology (MCBO)

Molecular mechanism of nutrient dependent plasma membrane remodeling

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Post-Doc

The ability to respond to growth factors and nutrient fluctuations in the environment is essential for cell growth and survival. A key response to these changes in the environment is the reconfiguration of the protein composition in the plasma membrane (PM). This involves ubiquitination of PM proteins and their subsequent endocytosis from the plasma membrane.
Little is known how the ubiquitination and endocytosis of nutrient transporters is regulated in response to changes in the cellular environment, despite their essential role in cellular homeostasis.
Our fundings suggest that nutrient limitation induces global and selective remodeling of the plasma membrane proteome, including the endocytic down-regulation of many different nutrient transporters. Here we study the molecular mechanisms that control starvation-induced endocytosis of nutrient transportes using yeast as a model system.
Our preliminary results suggest that starvation-induced endocytosis of nutrient transporters differs in several key aspects from their endocytosis under nutrient replete conditions. It (i) seems to be suppressed by TORC1 signaling and it (ii) requires defined switches in ubiquitin ligase adaptors of the ART family (ii) which drive the assembly of distinct ART-Rsp5-ubiquitin ligase complexes that (iii) mediate a different ubiquitination code required for the endocytosis of nutrient transporters under different growth conditions.

Structure and function of the pistol ribozyme by solution NMR spectroscopy

Over the past few years, the knowledge about the important roles, that RNA and DNA play in cellular processes, has significantly grown. In the past, DNA has been regarded as a simple storage molecule of genetic information and RNA as a linker/translator between the DNA's information and proteins. Nowadays, this view is replaced by an unexpected wealth of additional and novel functions especially associated to RNA. There is a need for investigations addressing the structure and dynamics of nucleic acids and how function is encoded in these properties of a biomolecule. The presented research focus on a catalytically active RNA – the pistol ribozyme – by solution NMR spectroscopy. Thereby, unprecedented insights on the interplay of structure and dynamics to fulfil the catalytic function are obtained.
Our group has strongly focused on the synthesis of atom-specific labelled RNA phosphoramidites and their incorporation into RNA. These building blocks are modified with 13C and/or 15N isotopes allowing state-of-the-art NMR experiments on RNA. The chemical solid phase synthesis makes site-specific labelling possible, which is not amenable with any other existing RNA production method.
Recently, we could also show that the novel RNA isotope labelling protocol is especially useful in studying dynamic properties of nucleic acids. A site-specific deuteration step allowed the application of so called proton relaxation dispersion experiments by reducing the size of homonuclear scalar couplings. Thereby, high energy conformations of the pistol ribozyme, which are populated to a low degree (< 10%) can be studied in unprecedented detail and hopefully high resolution 3D structures of this fleetingly populated states will become amenable soon.

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PhD programme
Center for Molecular Biosciences Innsbruck (CMBI)

Poster # 25

Biochemistry and Cell Biology

Investigating the BCL-2 protein family in mitotically arrested breast cancer cell lines

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In the last decades apoptosis, a regulated form of cell death has become more and more investigated and became an important target in treating cancer. The specific induction of apoptosis in cancer cells is arguably the main goal of most chemotherapies. The B-Cell lymphoma (BCL-2) protein family is the most critical regulator of apoptosis and consists of pro- and anti-apoptotic proteins which interplay with each other. Targeting the pro-apoptotic proteins with BH-3 mimetics and additionally inhibiting the cell cycle with microtubule-targeting agents (MTA) like Taxol can be used to specifically induce apoptosis in cancer cells.
The degradation of the anti-apoptotic MCL1 during mitotic arrest appears to be the molecular mechanism behind the mitotic death. We could show that the pro-apoptotic NOXA protein mediates the degradation of MCL1 during extended mitotic arrest. I am now investigating this NOXA/MCL1 axis in different breast cancer cell lines.
After analyzing the BCL-2 protein expression of breast cancer cell lines I also tested their sensitivity to Taxol (Paclitaxel) in combination with several BH-3 mimetics (ABT-737, ABT-199, UMI-77, Wehi-539) using an MTT assay. Thereby, I could show that although different cell lines react differently to the agents the most striking effects where visible using ABT-737 (targeting BCL-2, BCL-XL, BCL-W) or Wehi-539 (targeting BCL-XL) in combination with Taxol. So far, the two breast cancer cell lines I tested in more detail showed that MCL1 and NOXA are slightly degraded during extended mitotic arrest which confirms that the NOXA/MCL1 axis is present also in breast cancer cell lines.

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Master-Student

Establishing a platform for tumor organoids: one step toward precision cancer medicine

Poster # 26

Biochemistry and Cell Biology

Therapeutic strategies that are based on the individual genetic profile of a patient represent a new frontier of applied cancer research. These strategies are expected to reduce the socio-economic impact of current cancer therapies that are cost-intensive and often ineffective, thus releasing pressure on regional health systems. Especially in cancer research, standard cell culture conditions fail to proper mimic the parental tumor architecture and microenvironment. In this context, tumor-organoids are of special relevance. Tumor-organoids are three-dimensional cellular complexes that are cultivated in vitro from the cells that were obtained from patient tumors or biopsies. Tumor-organoids have the special property to mirror the key-features of the original patient's tumor with its microenvironment. Thus, Tumor-organoids will be an ideal tool to identify patient-specific therapies by performing drug-screenings on primary patient material. The aim of my work is to develop strategies for the cultivation and long term storage (Live-biobank) of patient-derived Tumor-organoids. For the future this Live-biobank and know-how will be accessible to the academic, translational, clinical and pharmaceutical research and development sector and will help to develop new strategies for precision cancer medicine.

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PhD programme
Molecular Cell Biology and Oncology (MCBO)

Towards the chemical synthesis of native stable isotope modified tRNAs for NMR spectroscopy

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One goal of the here presented work was to perform solid phase synthesis of RNA oligonucleotides using 2'-O-(2-Cyanoethoxymethyl) (CEM) protected phosphoramidites. This rather new method with its advantages, like higher coupling efficiency and efficient deprotection procedures, could lead to superior results for synthesizing long RNA sequences suitable for biophysical studies, e.g. NMR spectroscopy.
Additionally, stable isotope labeled dihydrouridine (DHU) building blocks - a common natural modification occurring in tRNAs – compatible with the CEM method were synthesized. Based on its altered structural and chemical features compared to uridine, a decrease in the structural stability can be expected and DHU can act as a dynamic hot spot introducing functional flexibility in tRNAs.
To corroborate this assumption, the four standard nucleosides as well as isotope labeled ones and dihydrouridine building blocks were synthesized as protected phosphoramidites to produce RNA sequences for nuclear magnetic resonance spectroscopic (NMR) investigations.
In an early stage, the CEM method was applied to synthesize a 48 nucleotide long tetramethylrhodamin aptamer and the method was compared with the 'standard' TOM method.
Finally, preliminary NMR experiments to address the structural and functional characteristics of dihydrouridine were carried out on a well investigated 14 nucleotide (nt) long hairpin RNA sequence. The base pairing property of DHU was examined with a 1H,15N,-HSQC experiment and its dynamics on a millisecond time scale within a double helical arrangement was probed via a 15N CPMG relaxation dispersion experiment.

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PhD programme
Center for Molecular Biosciences Innsbruck (CMBI)

Advanced cellular models for Alzheimer's disease by reprogramming and organoid formation

Alzheimer's disease (AD) is a chronic neurodegenerative disorder and is the cause of about 70% of dementia. An early symptom of AD is short-term memory loss and with advancing age includes language problems and disorientation, ultimately resulting in not managing self-care. The disease progress is associated with amyloid-β plaques and neurofibrillary tangles in the brain. There are two forms of AD defined by the age of onset. The familial or early-onset AD starts before 65 years of age and the sporadic or late-onset AD starts later than 65 years of age. More than 70% of Alzheimer's cases are caused by genetic risk factors.
Despite decades of intensive investigations the causative pathophysiological mechanisms of AD are still not well understood. Conventional cell culture models and animal models fall short to recapitulate all pathological hallmarks of AD in humans. The availability of induced pluripotent stem cells (iPSCs) derived from patient specific fibroblasts and 3D differentiation protocols have opened new possibilities to model diseases in vitro using human cells.
Here we used human iPSCs derived from Alzheimer patients carrying a deletion in exon 9 of the presenilin 1 gene (PS1ΔE9), a known risk factor for early onset or familial AD, isogenic controls from the same patient, where the PS1ΔE9 mutations has been repaired using CRISPR/Cas9, and controls derived from healthy patients without any symptoms of AD. We used these iPSC lines to generate cerebral cortical neurons and neural networks in an adherent 2D system, 3D Matrigel thin-layer differentiations and self-organizing 3D cerebral organoid to identify disease phenotype and new therapeutic targets in AD. Comparative analysis of cellular and molecular validation of AD cell models will be presented enabling further insight into AD pathophysiology.

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Post-Doc

Poster # 29

Biochemistry and Cell Biology

LAMTOR-complex in regulation of fat metabolism

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PhD programme
Molecular Cell Biology and Oncology (MCBO)

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 early embryonic-lethal in mice, but conditional knockouts 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.
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 in chow diet. Under fasting and re-feeding conditions of AdipoqLAMTOR2 -/- mice, a defect in activating mTORC1 signaling in BAT was observed. Although thermogenesis seems to be affected in AdipoqLAMTOR2 -/- mice, cold treatment reverses the phenotype observed in BAT and blood. The defect in mTORC1 activation is still present after cold exposure.
In summary, an adipose tissue specific knock out of LAMTOR2 disrupts BAT homeostasis and has effects on the whole body lipid metabolism.

Investigation of the enzymatic mechanism and the reaction kinetics of a prokaryotic RNA methyltransferase using NMR spectroscopy

Poster # 30

Biochemistry and Cell Biology

We present an NMR spectroscopic investigation of the bacterial methyltransferase RlmJ, which methylates the amino group of adenine 2030 in 23S rRNA during the early stages of the 50S subunit assembly in E.coli. The binding characteristics of substrate and co-factor analogues, the reaction mechanism and kinetic parameters of the SAM-dependent methylation are investigated using line-shape analysis of Methyl-TROSY experiments, CPMG relaxation dispersion and real-time NMR experiments. Substrate analogues are prepared by RNA solid-phase synthesis in order to probe the binding efficiency of the methyltransferase to these altered oligonucleotides and to explore the interactions in the binding pocket at an atomic level. The multi substrate enzyme kinetics of RlmJ are investigated by real-time NMR experiments employing Michaelis-Menten like diagrams.

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PhD programme
Chemistry

The cytochrome b5 CybE is regulated by iron availability and is crucial for azole resistance of *A. fumigatus*

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PhD programme
Gene Expression

Cytochrome P450 enzymes (CYP) play essential roles in redox metabolism in all domains of life including detoxification reactions and sterol biosynthesis. The activity of CYPs is fuelled by two electron-transferring mechanisms, heme-independent CYP reductase (CPR) and the heme-dependent cytochrome b5 (CYB5)/cytochrome b5 reductase (CB5R) systems. In this study, we characterised the role and regulation of the cytochrome b5 CybE in Aspergillus fumigatus. Deletion of the CybE encoding gene (cybE) caused a severe growth defect in two different A. fumigatus isolates, emphasising the importance of the CB5R system in A. fumigatus, while the non-essentiality of cybE indicates the partial redundancy of the CPR and CB5R systems. Interestingly, the cybE loss-of function caused growth defect was even more drastic in A. fumigatus strain Afs77 compared to strain A1160P+ indicating strain-dependent degree of compensation, which is supported by azole resistance studies. In agreement with CybE being important for assistance of the ergosterol biosynthetic CYP Cyp51A, deletion of cybE decreased resistance to the Cyp51A-targeting antifungal voriconazole and caused accumulation of the ergosterol pathway intermediate eburicol. Overexpression of cybE did not affect azole resistance demonstrating that the normal CybE amount does not limit Cyp51A activity. Expression of cybE was found to be repressed during iron starvation by the iron-regulatory transcription factor HapX demonstrating iron dependence of CybE not only at the level of enzyme activity but also at the level of gene expression.

Effect of nuclear magnetic resonance on the circadian clock and the hypoxia signaling pathway

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PhD programme
Biology

The circadian clock and the hypoxia signaling pathway interact bidirectionally, and both are important targets for several diseases such as osteoarthritis and cancer. Beside negative effects of electromagnetic fields on living organisms, in the last decades researchers concentrated on health benefits and demonstrated positive effects after specific application of electromagnetic fields. In this study, we therefore analyzed the effect of a 0.4 mT nuclear magnetic resonance (NMR) field on the zebrafish fibroblast cell line Z3, particularly on the circadian clock and the hypoxia signaling pathway. Our results revealed an increased circadian rhythm amplitude of cryptochrome1 in the NMR group together with a significantly increased gene expression of period1 and the major regulator of the hypoxia signaling pathway, hif1. Furthermore, protein expression of Peroxiredoxin-SO3 was decreased after 4 hours of NMR exposure in contrast to 4 x 1 h NMR treatment on four consecutive days where protein expression was increased. These results indicate an effect on the reactive oxygen species defense. In conclusion the data demonstrate that NMR interacts with the circadian clock and the hypoxia signaling pathway in a dose and time dependent manner.

Poster # 33

Biochemistry and Cell Biology

Secondary structure probing of the Hatchet ribozyme by site-specific 15N-labeling and solution NMR-spectroscopy

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Master-Student

We present a fast, reliable and economic synthetic access to 15N1-guanosine and -adenosine, and also 15N3-uridine and cytidine RNA phosphoramidite building blocks. These compounds were used for atom- and site-specific labeling of RNA constructs with sizes up to 80 nucleotides. The main focus was on folding studies of ribozyme RNAs. Novel ribozyme classes such as the hatchet ribozyme were recently reported.
For some of these novel ribozymes high resolution structures are available, but for the hatchet ribozyme structural information is rare limited to a secondary structure proposal. Thus, the 15N-labeled phosphoramidites were utilized to probe secondary structure elements of the hatchet ribozyme using state of the art NMR experiments. Some of the postulated secondary structure features could be confirmed, other base pairing interactions, however, could not be observed.
Currently, a rigorous NMR study is carried out to establish a full secondary structure proposal for the hatchet ribozyme, a crucial pre-requisite for a full high resolution 3D structure elucidation.

RAF inhibitors allosterically elevate mutated RAS:RAF complexes

Poster # 34

Biochemistry and Cell Biology

Oncogenic mutations in RAS GTPases or BRAF kinases deregulate the integrity of their binary interactions leading to hyper-activation of MAP-kinase signaling. The most common oncogenic BRAF variant contains the V600E mutation and has become the prime target for therapeutic intervention showing anti-tumor responses e.g. in metastatic melanoma patients. However, therapeutic effects are often temporary and proposed drug resistance mechanism involve alterations of molecular interactions of the RAS-RAF-ERK cascade provoking MAP-kinase reactivation. To systematically correlate BRAFV600E inhibitor (BRAFi) efficacies with RAS:RAF complex formation we implemented a luciferase-based intermolecular protein-fragment complementation assay (PCA) platform. We used an extendable collection of RAS and RAF protein variants to directly probe consequences of small molecule:kinase interactions. Using the PCA reporter as quantitative read-out for cellular protein:protein interactions we approved that GTP-loading of H/N/K-RAS significantly elevated complex formation with full length BRAF. Next, we recorded the dose- and time-dependent effect of ATP-competitive small molecule inhibitor binding to distinct cellular BRAF complexes in vivo. Notably, we report an allosteric boosting effect of the selective and effective first generation BRAFi vemurafenib, dabrafenib, and encorafenib on defined RAS:BRAFV600E complexes. We propose that the direct BRAFi driven transformation of binary RAS:BRAFV600E interactions contributes to the incidence of paradoxical MAP-kinase activation and drug resistance observed in BRAF-mutant tumors.

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PhD programme Biology

First steps towards understanding ESCRT-•-III capping

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PhD programme
Molecular Cell Biology and Oncology (MCBO)

Organelle biogenesis and maintaining subcellular compartmentalization of eukaryotic cells requires membrane modeling processes.
A multi subunit molecular machinery, called the endosomal sorting complexes required for transport (ESCRT) drives the formation of multivesicular bodies (MVB), membrane abscission at the end of cytokinesis, HIV budding, nuclear envelope closure and maintenance for the repair of holes in the plasma membrane. Common denominators in all those reactions are the AAA ATPase Vps4 and the subunits of the ESCRT-III complex.
ESCRT-III is a heterooligomer consisting of the four proteins Vps20, Snf7, Vps24 and Vps2. Vps20 nucleates and triggers Snf7 homopolymerization, and this is capped by Vps24 and Vps2. In vitro models show that binding of Vps24 and Vps2 to Snf7 deform the protein from a filament in a three dimensional spiral. This allows binding of the AAA Vps4, but how this contributes to ILV formation is unclear.
As a termination on the forming ESCRT-III filament, the capping factors Vps24 and Vps2 have to bind to Snf7 to recruit Vps4. Although it was shown that this binding is necessary for the constriction of the neck of forming ILV, how the capping factors Vps24 and Vps2 bind to each other and to the Snf7 filament is poorly understood.
Our goal is to understand how Vps24 and Vps2 bind to each other and therefore, how the of the capping structure is organized, and how they are recruited to the Snf7 filament, and therefore allow Vps4 to be recruited to the forming ILV site.
By using yeast as the best-suited model organism, we will combine genetics, live cell imaging, immunoprecipitation and cross-linking mass spectrometry identify the binding within these proteins.
Additionally we will use purified ESCRT subunit to reconstitute the ESCRT machinery on artificial liposomes to further study the binding in vitro, cross-link the peptides and determine the interactions by mass spectrometry.
Altogether, we expect to get a better understanding how Vps24 and Vps2 bind to each other and to Snf7, terminating the ESCRT-III complex formation and recruiting Vps4, which leads to the abscission of the ILV and to a disassembly of the complex.

Discovery of the first dual inhibitor of 5-lipoxygenase-activating protein and soluble epoxide hydrolase using pharmacophore-based virtual screening

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Post-Doc

Leukotrienes (LTs) are pro-inflammatory lipid mediators derived from arachidonic acid (AA) with roles in inflammatory and allergic diseases. The biosynthesis of LTs is initiated by transfer of AA via the 5-lipoxygenase-activating protein (FLAP) to 5-lipoxygenase (5-LO). FLAP inhibition diminishes LT formation exerting anti-inflammatory effects. The soluble epoxide hydrolase (sEH) converts AA-derived anti-inflammatory epoxyeicosatrienoic acids (EETs) to dihydroxyeicosatetraenoic acids (di-HETEs). Its inhibition consequently counteracts inflammation. Targeting both LT biosynthesis and the conversion of EETs with a dual inhibitor of FLAP and sEH may represent a novel, powerful anti-inflammatory strategy. The aim of this study was to discover compounds dually inhibiting FLAP and sEH as novel pharmacological tools and potential starting points for the development of new anti-inflammatory drugs.
Ligand based pharmacophore models for FLAP were generated in LigandScout version 3.2 based on 11 highly active inhibitors from literature. The Specs commerical database was virtually screened with these models and the hit compounds were screened with sEH inhibitor models generated in a previous study. After inspection of the virtual hits, 20 compounds were selected and tested in a cell-based FLAP test system and a cell-free sEH activity assay .
Out of the 20 test compounds, five compounds were active on FLAP and four on sEH. Among them, the first dual inhibitors for sEH and FLAP were identified. The most potent compound was N-[4-(benzothiazol-2-ylmethoxy)-2-methylphenyl]-N'-(3,4-dichlorophenyl)urea with IC50 values of 200 ± 40 nM for FLAP and 20 ± 7 nM for sEH, respectively.
These promising results showcase the impressive predictive power of pharmacophore modeling to identify dually acting inhibitors within the AA cascade. The most potent compound is currently under further investigation and serves as a lead structure in a SAR study on FLAP/sEH dual inhibition.

Poster # 37

Biochemistry and Cell Biology

From Substrate Specificity to Small Molecule Specificity

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PhD programme
Chemistry

Protease substrate profiling has nowadays almost become a routine task for experimentalists and the knowledge on protease peptide substrates is easily accessible via the MEROPS database. We present a shape-based virtual screening workflow using vROCS that applies the information about the specificity of the proteases to find new small molecule inhibitors. Peptide substrate sequences for 3-4 substrate positions of each substrate from the MEROPS were used to build the training set. 2D substrate sequences were converted to 3D conformations through mutation of a template peptide substrate. The vROCS query was built from single amino acid queries for each substrate position considering the relative frequencies of the amino acids in the protease peptide substrates collected in the MEROPS. The peptide substrate based shape-based virtual screening approach gives good performance for four example protease targets, thrombin, factor Xa (fXa), factor VIIa (fVIIa) and caspase-3 (casp-3) with the DUD-E dataset. The results show that the method works for protease targets with different specificity profiles as well as for targets with different active site mechanisms. As no structure of the target and no information on small molecule inhibitors are required to use our approach, the method has significant advantages in comparison with conventional structure- and ligand-based methods.

Loss of PTEN in prostate cancer cells leads to a switch to succinate-mediated mitochondrial respiration associated with increased expression of NaDC3 and HIF-1alpha

Poster # 38

Biochemistry and Cell Biology

Metabolic changes are a hallmark of prostate cancer cells, mainly resulting in significant alterations of glycolysis and fatty acid synthesis pathways. In order to reveal novel targets that could be used for therapeutic intervention, we performed a comprehensive metabolic analysis of different human (LNCaP, DuCaP, PC-3, DU145) and murine prostate cancer cell (PCa) lines.
In line with previous studies we found that loss of phosphatase and tensin homolog (PTEN), a major driver of prostate cancer cells, is associated with high glycolytic activity, increased lactate production and elevated expression of hexokinase 2 (HK2). This increased glycolytic activity corresponds with weak activity of pyruvate dehydrogenase (PDH), which drives pyruvate into oxidative phosphorylation, and high expression of the PDH inhibitor PDK1 (pyruvate dehydrogenase kinase). Mitochondrial routine respiration was significantly elevated in all human and mouse PTEN- compared to PTEN+ cells. While digging deeper into these pathways we showed that high lactate production in PTEN- cells leads to a switch in substrate-induced mitochondrial respiration towards complex II. In particular we found that PTEN- PCa cells favour succinate as substrate for oxidative phosphorylation (complex II) while lowering the capacity to use glutamate and pyruvate (complex I). In addition, we found that the Na(+)-dependent dicarboxylate transporter NaDC3, that is responsible for succinate uptake into cells, is elevated in PTEN- compared to PTEN+ cells and that HIF-1alpha, a molecule that is stabilized by succinate, is increased in cells lacking PTEN. Downregulation of NaDC3 using siRNA, causes a decrease in cell viability, lactate production and HIF-1alpha protein expression.
Our data indicate that uptake of succinate via the NaDC3 transporter could enable PTEN- PCa cells to fulfil increased energy requirements and that intervening with this pathway may offer a new way for treatment of PCa.

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PhD programme
Molecular Oncology

Poster # 39

Biochemistry and Cell Biology

Purification, crystallization, and assessment of substrate specific activities and inhibition of fumarylacetoacetate hydrolase (FAH) domain containing proteins (FAHD)

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Post-Doc

The observation of the existence of eukaryotic oxaloacetate decarboxylases was first described in the late last century, in rat liver homogenate. The very enzyme beyond these observations remained unclear, however, until recent work of our group has identified fumarylacetoacetate hydrolase domain containing protein 1 (FAHD1) as a mitochondrial acylpyruvase in general and oxaloacetate decarboxylase in eukaryotes. The conserved FAH fold has been associated with important β-diketone hydrolase reactions and the deficiency of FAHD1 is by now associated to various phenotypes, ranging from locomotion deficiencies in C. Elegans, over indications of body fat related and neurological disorders and neurodegenerative diseases in mice, to hereditary tyrosinemia type I in humans. We recently managed to establish a series of assay strategies on the 96-well UV transparent plate in order to measure the enzyme catalyzed decomposition of oxaloacetate with much lower amounts of enzyme. This appears to be a major advantage as activities and Michelis-Menten kinetic descriptors (vmax, KM) can be assessed of enzymatic variations (mutations, etc.) and homologous structures that may not be as easily obtained and purified in large quantities, as is the case with the human FAHD1 wild-type. Recently created highly resolved crystal structures enabled us to investigate FAHD1 more closely. This will help us to understand the catalytic reaction mechanism of the FAH fold and to develop nanomolar inhibitor compounds for the human FAHD1 protein eventually.

Hunting for the glue: temporary adhesion of the flatworm *Macrostomum lignano*

Poster # 40

Biochemistry and Cell Biology

Biological adhesion is widely spread among the animal kingdom. The adhesives secreted by marine invertebrates show remarkable performances providing great potential for future biomedical and industrial applications. Whilst permanent adhesive systems like mussels, barnacles, and annelids have been investigated into detail over the last couple of years, little is known about the adhesive substances used for temporary, reversible adhesion often found in echinoderms and flatworms. We showed that the adhesion of the free-living, marine flatworm Macrostomum lignano, relies on the secretion of two large proteins called Mlig-ap1 and Mlig-ap2, both comprised in the same vesicles. Bioinformatical analysis of the two proteins revealed their enormous size of 4,535 and 9521 aa, respectively and their highly repetitive structure. Successful knockdown of the two mRNAs via RNAi revealed, that both proteins are essential for the adhesion of M. lignano. We could further show, that glycosylation as posttranslational modification of at least one of the two proteins plays an important role. Polyclonal antibodies raised against the two proteins combined with lectin staining allowed us to elucidate the spatial distribution of the secreted material. The obtained data provide the first identification and functional characterization of two major adhesive components in a flatworm species. With this study we pave the way for future investigations and render an important step towards a new, bio-inspired adhesive.

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PhD programme Biology

Poster # 41

Biochemistry and Cell Biology

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

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PhD programme
Molecular Cell Biology and Oncology (MCBO)

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 a recently described BORC complex, which has been linked to endosomal positioning and biogenesis of lysosomes and lysosomal related organelles.
In order to dissect the involvement of the BORC complex in endosomal biogenesis, we have generated a CRISPR/Cas9 mediated knock out (KO) of a core BORC component C10orf32 in the largely diploid fibrosarcoma cell line HT1080. This deletion had little effect on mTOR and MAPK signaling, but resulted in an alteration of the late endocytic compartment morphology. Whilst the wild type (WT) cells were able to spontaneously build enlarged lysosome like vesicles, appearing to have only partly digested cargo on electron microscopy images, the KO cells seemed to have much compacter, denser lysosomes. This difference was most prominent upon treatment with lysosomal inhibitors such as Chloroquine or Bafilomycin A, which induced this process even further in the WT cells but not in the KOs. Interestingly we have not yet observed any defects in either homo- or heterotypic fusion of late endosomes/lysosomes, neither have we been able to identify any defects in these organelles, as in both cell lines they seemed to be targeted properly by dextran uptake and accumulated the acidophilic dye lysotracker.
Furthermore, we have observed a dramatic increase of the lysosomal associated membrane protein Lamp1, but not of its closely related Lamp2. Whether this is connected or even causative of the late endocytic phenotype observed in these cells would be the focus of further investigation.

Alpine plants contain phytochemicals that interact with intracellular pathways to delay aging

Poster # 42

Developmental Biology and Aging

Botanicals play an important role as pharmaceuticals and cosmeceuticals. Phytochemicals interact in different cellular pathways and counteract the onset of aging. The harsh habitat in which some plants grow and thrive, such as Alpine Plants, suggests that they have developed chemical defense mechanisms against environmental stressors, which have protective attributes towards cells.
Many of the stressors Alpine plants are exposed to, influence the onset of senescence in human cells. For the present study, we analyze 150 alpine plants for anti-aging properties. In our study, we give a particular attention on how reactive oxygen species (ROS) drive cells towards senescence and if this can be prevented by the use of Alpine phytochemicals. Therefore, we developed two cellular systems designed to investigate the protective attributes of phytochemicals towards ROS and ROS derived damage. These systems are based on the analysis of two proteins: (1) NADPH oxidase 4 (NOX4) is involved in determining the onset of replicative senescence by continuously producing ROS. Inhibition of NOX4 by phytochemicals can delay the onset of aging. (2) Proteasome activity is vital for maintenance of proteostasis. Exposure to tert-butyl hydroperoxide, a source of ROS, causes inactivation of the proteasome and induces the onset of senescence. Phytochemicals, that can reactivate the proteasome, can protect human fibroblasts from aging. Our data have revealed potential candidates that are able to inhibit NOX4 and/or reactivate the proteasome. These extracts will be fractionated to isolate the active compounds responsible for the anti-senescence activity.

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PhD programme
Biology

Molecular regulation of pancreatic islet formation

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PhD programme
Biology

Pancreatic islet cells emerge from the duct epithelium and undergo delamination, migration, and clustering to form mature islets in a process that is poorly understood due to the deep internal location of the pancreas in most vertebrate model organisms. Zebrafish is an in vivo system well suited for studies of islet cell assembly due to its transparency and the accessible location of the larval pancreas, which enables live imaging of dynamic migratory processes.

To determine the molecular mechanisms behind pancreatic islet formation in vivo, we used zebrafish larvae. With the help of in vivo time-lapse fluorescence microscopy, in combination with quantitative image analysis approaches, it is possible to visualize the dynamics of cell movements and determine regulating intracellular signaling pathways. In wild type embryos, endocrine cells showed complex morphologies, shape changes and moved together. To test different signaling factors for their function in pancreatic islet formation, chemical treatments and genetic approaches were used with which candidate signals could be inhibited and changes in movement and morphology of endocrine cells were examined. PI(3)K inhibition by wortmannin or LY294002 caused differences in cell shape and motility as compared to controls, in particular a decrease in formation of fine protrusions. In addition, we found that the volume of secondary islets is significantly reduced by PI(3)K inhibition. Furthermore, expression of PTX during islet assembly led to decreased secondary islet volumes, suggesting that PI(3)K and GPCR inhibition interferes with islet assembly in zebrafish.

For future experiments to define the endocrine-specific effects of modulating the above mentioned factors and to identify additional important regulators, an inducible, tissue-specific gene expression system is being established.

Beta-Catenin acts in an early, position-independent regeneration response in the simple eumetazoan Hydra

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PhD programme
Biology

The Wnt/β-Catenin pathway plays crucial roles in regenerative processes throughout eumetazoans It also acts in regeneration and axial patterning in the simple freshwater polyp Hydra. Previous studies have identified β-catenin as an early response gene in Hydra head regeneration. Here, we have studied the role of β-Catenin in more detail. A previously identified set of β-Catenin target genes show distinct expression patterns in the hypostome, in the tentacles, or in an apical gradient in the body column and ChIP experiments show Tcf-binding at their promoters. These genes are transcriptionally activated in the tips of early head regenerates until differentiated head structures appear. Furthermore, β-catenin and the target genes are unexpectedly upregulated in early foot regenerates and the transient expression starts to disappear after 12 hours. We propose that gene regulatory β-Catenin activity is generally required as an early regeneration response acting in wound healing and tissue reorganization.

In a complementary set of data, we show that inhibition of β-Catenin/Tcf interaction by the small molecule inhibitor iCRT14 blocks head as well as foot regeneration. In iCRT14-treated animals regeneration is blocked, but β-catenin and its targets are transcriptionally activated and remain upregulated in both head and foot regeneration. This indicates that an initial phase of position-independent gene activation may be followed by position-specific programs in order to proceed forming head or foot structures. Hydra brachyury1, another early response gene of head and foot regeneration, remains expressed in regenerating heads but disappears after about 12 hours from foot regenerates. In contrast to the mRNA level, the HyBra1 protein appears with strong delay in cell nuclei in head regenerating tips - about 12 hours after decapitation. However, foot regenerates never show translation of HyBra1 protein.

In summary, we propose that Hydra regeneration starts with general, position-independent activation of fast responding genes, and β-Catenin is a core component of this early regulatory network. After wound healing, head- and foot-specific molecular programs take over and regulate regeneration of corresponding structure. Our data suggest that translational control mechanisms may play a decisive role in this second phase, and HyBra1 is an excellent candidate for such a regulator of head specification.

Poster # 45

Developmental Biology and Aging

Organ specific gene expression in the regenerating tail of *Macrostomum lignano*

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PhD programme
Biology

Temporal and spatial characterization of gene expression is a prerequisite for the understanding of cell-, tissue-, and organ-differentiation. In a multifaceted approach to investigate gene expression in the tail plate of the free-living marine flatworm Macrostomum lignano we performed a posterior-region-specific in situ screen, RNA-seq of regenerating animals, and a functional analysis of selected tail-specific genes. The in situ screen revealed transcripts expressed in the antrum, cement glands, adhesive organs, prostate glands, rhabdite glands, and other tissues. Next we used RNA-seq to characterize temporal expression in the regenerating tail plate. The RNA-seq data revealed a temporary restricted onset of the regeneration of the e.g. adhesive organs and the copulatory apparatus. In addition, we identified three novel, not annotated genes that are solely expressed in the regenerating stylet. RNA interference showed that these genes are required for the formation of not only the stylet, but the whole male copulatory apparatus. RNAi treated animals lacked the stylet, vesicula granulorum, seminal vesicle, false seminal vesicle, and prostatic glands, while the other tissues of the tail plate like adhesive organs regenerated in a normal way. In summary, our findings provide a large resource of expression data during homeostasis and tail regeneration and pave the way for a better understanding of organogenesis in M. lignano.

Poster # 46

Developmental Biology and Aging

Molecular Regulation of the oncogenic miRNA-17-92 cluster

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PhD programme
Molecular Oncology

MicroRNAs are a class of small non-coding RNAs that posttranscriptionally regulate gene expression by sequence-specific repression of mRNA. Since almost every cellular pathway is fine-tuned by miRNAs, they form an essential regulatory layer in multicellular organisms. However, due to their widespread regulatory potential, it is also not surprising that aberrant expression of miRNAs is often correlated with human pathologies such as cancer.
The polycistronic miR-17-92 cluster, which encodes 6 individual miRNAs (miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1, miR-92a-1) in one long primary transcript, is one of the first described miRNA genes with a clear oncogenic role in different types of human cancer.
Interestingly, recent data indicate that the cluster members do not equally contribute to the progression of cancer as previously suggested. In contrast, it seems that individual members can confer oncogenic or tumor suppressive functions. This suggests that not the overexpression of the cluster per se, but the imbalanced expression of individual cluster members may be a critical contributor to tumor development. Indeed, sequencing efforts in cancer patients have identified miR-17-92 signatures that favor expression of either oncogenic or tumor- suppressive cluster members. However, little is known about how such an imbalanced expression of miRNAs within a cluster is established on the molecular level, warranting an in-depth investigation.
We established the workflow for a genome-wide CRISPR/Cas9 loss-of-function screen aimed to identify novel regulators of the miR-17-92 cluster. In short, cells expressing fluorescence-based reporters that allow the quantification of miR-19b and miR-92a activity were transduced with a CRISPR library comprising about 120.000 small guide RNAs (sgRNAs) targeting all coding genes. Cells exhibiting altered miRNA activity as measured by flow cytometry were isolated by cell sorting and the corresponding sgRNAs were retrieved by next generation sequencing and thorough bioinformatics data analysis. Several of those candidates we partially reviewed, but these need further confirmation and validation experiments.

Antigen-experienced immune cells in the human bone marrow and the impact of ageing, senescence and Cytomegalovirus

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The bone marrow (BM) is known to be the site of hematopoiesis, but it has also been shown that antigen experienced immune cells migrate back to the BM, where they can remain in bone marrow niches for an extended period of time. Previous studies demonstrated an accumulation of highly differentiated CD8+CD28- T cells in the aging BM, however the impact of this subpopulation on other cell subsets has not yet been described.
In this study bone marrow mononuclear cells (BMMC) were isolated from human BM samples using collagenase digestion and density gradient centrifugation. Lymphocyte subpopulations were analyszed by flow cytometry.
The number of CD8+CD28- T cells which correlates positively with age, negatively correlates with IgM Memory B cells (CD27+IgD+CD19+), and positively correlates with CD4 Effector Memory cells (CD45RO+CCR7-). Latent infection with Cytomegalovirus (CMV) is known to enhance age-related changes of the T cell pool, such as the accumulation of highly differentiated effector T cells. We therefore included the serostatus for CMV in our analysis. One hallmark of highly differentiated T cells is the production of pro-inflammatory cytokines such as IFN-γ and TNF-α. We demonstrate a higher production of these cytokines in BMMC from CMV-seropositive donors in response to unspecific stimulation with PMA and Ionomycin. Several markers of cellular senescence were analysed by flow cytometry in order to further characterise highly differentiated T cells in the human BM.
These preliminary data suggest that immunological bone marrow niches offer limited space and that accumulation of one cell type, such as highly differentiated CD8+ T cells, affects the immune cell composition in the aging BM. Further investigation will focus on how these limitations are set and whether the specificity of these antigen-experienced cells affects their ability to migrate back to the BM.

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PhD programme
Host Response in Opportunistic Infections (HOROS)

G-Protein Coupled Receptors in Early Zebrafish Development

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G-protein coupled receptors (GPCRs) are the largest vertebrate protein family. They mediate a plethora of different processes via many different downstream signalling mechanisms. Recently, GPCRs besides the main Hedgehog (Hh) mediator Smoothened have been implicated in positive and negative control of Hh signalling through regulation of the cAMP-dependent Protein Kinase A (PKA) activity at the primary cilium, the main organelle in Hh signalling. Hh signalling is an important factor in embryonic patterning, cell fate specification, proliferation and migration.
It is very likely that the recently described GPCRs 161 and 175 are only a few among many GPCRs that are part of the Hh signalling pathway. Additional GPCRs might modulate different aspects of Hh signalling in a tissue-specific manner. We plan to find additional GPCRs involved in the control of Hh signalling and test their regulation of PKA by employing a transgenic zebrafish reporter line for an in vivo luciferase complementation assay, which allows a sensitive real time readout of PKA activity. The biological relevance of candidate receptors will be assayed by functional and genetic studies in zebrafish by studying their expression and subcellular localization as well as overexpression and knockout phenotypes.
These results will provide insights into additional layers of Hh signalling regulation and biological processes it controls, like embryonic development and the onset and progression of aggressive tumors like medulloblastoma, aiding a deeper understanding of the basic principles governing these processes and allowing the discovery of new drug targets.

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PhD programme
Center for Molecular Biosciences Innsbruck (CMBI)

The PIDDosome controls polyploidization during liver development and regeneration

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PhD programme
Molecular Cell Biology and Oncology (MCBO)

Caspase-2, the most conserved member of the caspase family, has been implicated in several physiological processes ranging from control of metabolism to the DNA damage responses and maintenance of genome integrity. However, the role of caspase-2 is still controversial and, to date, little is known about its substrates or cellular events triggering activation. As caspase-2 has been proposed to be involved in maintenance of genome integrity, we assessed the function of caspase-2 following failed cell division and were able to identify supernumerary centrosomes as a selective trigger for PIDDosome-dependent caspase-2 activation, resulting in p53-mediated cell cycle arrest.
In several mammalian tissues such as the heart or liver, cytokinesis failure is part of normal organ development. Hepatocytes, for example, increase their polyploidy over the organism's life span, either via incomplete cytokinesis or endoreduplication, both resulting in cells carrying additional centrosomes. Hence, the liver poses as an ideal model to study the role of caspase-2 during programmed polyploidization in vivo.
In order to shed light on the role of caspase-2 in liver organogenesis, we analyzed hepatocytes of PIDDosome-deficient mice over time which displayed a significant ploidy increase when compared to the wild type animals. Since hepatocytes are prone to cytokinesis failure in periods of extensive liver growth, postnatally or during tissue regeneration, we focused on proliferating hepatocytes during normal development as well as after partial hepatectomy. We found PIDDosome-dependent caspase-2 activation to be required to restrict the degree of polyploidization in both developing and adult hepatocytes during regeneration. Our results clearly demonstrate for the first time that caspase-2 plays a key-role in the regulation of hepatocyte ploidy during both liver development and regeneration.

Genome editing strategies to study the pancreatic lineage determinant MNX1 in ‚in vitro' differentiated human beta-cells

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PhD programme
Biology

MNX1 is a homeobox-transcription factor with highly conserved critical functions in neuronal and pancreatic cell specification. In the context of pancreas development, MNX1 plays essential roles in early organ morphogenesis and during specification, maturation and maintenance of insulin producing beta-cells. Despite its relevance for Diabetes research, very little is known about the underlying molecular mechanisms of these MNX1 activities and currently no direct molecular target of MNX1 had been described.
In order to understand the molecular functions of MNX1 in the context of human pancreatic development, a project using stem-cell derived mutated human beta-cells was initiated. As part of these studies, we established CRISPR/Cas9 genome-editing methods for generating two types of MNX1 knock-in stem cell lines. Initial tests were done in HEK cells to select sgRNAs allowing efficient indel generation and to test screening approaches for identifying clones with proper integrations of the knock-in constructs by homology directed repair. In particular, we aim for the generation of a cell line expressing a fully functional but epitope-tagged MNX1 protein together with the in vivo reporter GFP under control of endogenous MNX1 enhancer. The GFP-expression will highlight MNX1 activity while the tag will allow ChIP-based identification of direct MNX1 targets. The second line will be used to study loss-of-function phenotypes. As MNX1 has multiple roles during beta-formation, we aim for a conditional knock-out allele in which MNX1 can be inactivtaed at every developmental stage of the pancreas. For this line we are currently testing FLEx-strategies for Cre/LoxP based uni-directional DNA-inversion leading to a truncated and GFP tagged inactive MNX1 protein. Recent results will be presented.

Investigating the Role of Gpr161 in Hedgehog Signalling

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PhD programme
Center for Molecular Biosciences Innsbruck (CMBI)

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 signalling 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 locality where PKA regulation occurs is still unclear. Recently it was shown that the orphan G-protein coupled receptor Gpr161, a negative regulator of Hh signalling, localises to the primary cilium, activates PKA, and exits the cilium upon Hh pathway activation. In a first groundwork study we have shown that Gpr161 is both a target of PKA and that it acts to localise PKA to the primary cilium, suggesting that Gpr161 plays a central role in the regulation of PKA activity downstream of Hh signalling.
The aims of this project are to study how a loss of Gpr161 effects vertebrate development using CRISPR/Cas9 induced gene knockouts in zebrafish and to gain an understanding about how the interaction of Gpr161 and PKA influence Hedgehog Signalling on a cell biological level.

Role of mnx1 in zebrafish endocrine cell fate determination

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Post-Doc

Mnx1 is a mainly repressive homeobox transcription factor with conserved roles in motoneuron formation, pancreas morphogenesis and β-cell differentiation. How Mnx1 conducts its fate-determining functions is currently not known.
Recent work in fish and mouse showed that loss of β-cells in mnx1 deficient animals is caused by fate conversion of β-cell progenitors either into α- or δ-cells, respectively. Based on these findings it was proposed that Mnx1 supports β-fate implementation by repressing α- or δ-fate determinants. This model predicts that an ectopic activation or repression of Mnx1-targtes in the developing pancreas should result in the formation of more or less α-/δ-cells. By using transgenic animals for conditional activation of Mnx1, and a transcriptional activating form of Mnx1 (Mnx1-VP16) we tested this model. In contrast to the expected roles in defining a specific endocrine lineage, our findings suggest a role of mnx1 in the general progression of early endocrine differentiation. To enable more detailed analysis about the role of mnx1, we now generated loss-of-function mutants by using TALEN genome editing technology. Mnx1Δ31/ Δ31 fish serve as a model for addressing molecular and cellular requirements of Mnx1 in β-cell differentiation and maintenance. Mutant embryos show a strong decrease of Insulin expressing cells, while both Glucagon and Somatostatin positive cells are significantly increased. As Mnx1Δ31/ Δ31 fish are viable and reach adulthood we analyzed hormone expression in the mature organ. Mutant islets compensate for early β-cell reduction and show that the majority of cells are bihormonic and co-express Insulin and Somatostatin.
Recent data including cell-tracking analyses and molecular approaches for the identification of direct Mnx1-target genes will be presented.

Poster # 53

Developmental Biology and Aging

Bioadhesion in the Ascidian Ciona intestinalis

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PhD programme
Biology

Marine bioadhesion increasingly inspires biomimetics towards tissue compatible glues or antifouling compounds. Ascidian (tunicate) larvae produce adhesives for settlement and metamorphosis to sessile adults. We study adhesion of Ciona intestinalis, a well established model organism for developmental genetics featuring an invariable cellular lineage, a compact genome and rich experimental resources.. We could hereby find markers for the different cells types within the adhesive organ, notably two markers staining both, specific non-neural cells within the organ and adhesive plaques deposited on the substrate. Under high resolution microscopy, a meshwork of adhesive structure was observed in the plaques. Differential transcriptomics and proteomics have furthermore identified several candidate genes possibly contributing to ascidian glue formation. We now analyse their localised expression by in situ hybridization and their involvement in adhesion by gain- and loss-of-function assays using lineage specific overexpression and CRISPR or/and MO mediated knockdown. Adhesive forces will be analysed in the future.

Poster # 54

Genetics and Genomics

The mode of action of the *Penicillium chrysogenum* antifungal proteins PAF and PAFB: Membrane glucosylcermides are important susceptibility determinants in *Neurospora crassa*

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PhD programme
Gene expression

The small and cysteine-rich antifungal proteins PAF & PAFB from Penicillium chrysogenum have potent growth inhibition activities against filamentous pathogenic fungi and are not toxic to mammalian cells. These proteins therefore represent promising compounds for the development of novel antifungal treatment strategies. The detailed knowledge of their mode of action is a prerequisite for their future application in medicine and agriculture.
In previous studies we could show that the plasma membrane plays an important role in the interaction of PAF and PAFB with fungal cells. The aim of this study was to investigate in more detail the susceptibility determinants in the fungal membranes for PAF and PAFB.
We tested several Neurospora crassa mutants with defective lipid biosynthesis to investigate if membrane lipids regulate the susceptibility to PAF and PAFB.
In growth inhibition assays we could show that the deletion of genes coding for enzymes involved in glucosylceramide synthesis (ceramide synthase (lac1) and glucosylceramide synthase (gcs1)) results in a resistant phenotype of the respective N. crassa mutants in the presence of PAF, but not of PAFB. These data were further confirmed with fluorescence uptake studies, where the Δlac1 mutant showed no PAF uptake, whereas PAFB localized into the vacuoles and was then partly released into the cytoplasm after 8 h of exposure. In the Δgcs1 mutant, PAF was internalized into the vacuoles and remained trapped. PAFB, in contrast, localized to vacuoles and to the cytoplasm and the fungal cells showed clear signs of cell death.
We therefore conclude that the presence of glucosylceramide in the fungal membrane plays an important role in the interaction of PAF with the susceptible fungus, whereas PAFB seems to have a different mode of action which needs to be further investigated. However, ceramides are also second messengers and involved in fungal signal transduction in response to environmental stimuli. Therefore, we cannot exclude that the observed PAF-resistant phenotypes resulted from deregulated signalling in the N. crassa mutants. We further conclude that the toxic effects of PAF and PAFB seem to be triggered when the antifungal protein is released from vacuoles and reaches the cytoplasm.

CHD1 regulates metabolism and ageing of the fly

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PhD programme
Gene Expression

CHD1 belongs to the SNF2 family of ATPases involved in the regulation of transcription and chromatin functions. In Drosophila, Chd1 deletion results in shortened lifespan, a reduced number and viability of offspring, sterility, immunodeficiency and altered feeding behavior. Hence, in investigation for further factors contributing to the impaired phenotype of Chd1 mutants we decided to take a closer look on energy supply and utilization of flies in the absence of CHD1.
Here we focused on the analysis of the feeding behavior of Chd1 mutants under normal physiological conditions. We found that not only was food intake significantly reduced in adult flies in the absence of CHD1, but also mobility, orientation, gustatory and olfactory sensing, which are determining factors in food recognition, are severely impaired in the absence of CHD1. Additionally, Chd1 mutants show a significant impairment of carbohydrate and lipid metabolism and further analysis of the mechanisms that underlie the food restriction following Chd1 depletion pointed towards an impairment of metabolic signaling. Moreover, the fitness and lifespan of Chd1 deficient flies is significantly impaired. Thence we decided to further investigate the major intercalating signaling pathways orchestrating the fly´s fitness, the insulin/insulin-like growth factor (IlS) pathway and TOR pathway. Collectively, the here presented findings provide novel insights about the roles of chromatin remodelers, in specific CHD1 function, in metabolic processes that control longevity.

Igf1r knock-down impairs neonatal cardiac regeneration

By 2017 ischemic heart disease remains the number one killer worldwide. Whereas adult hearts lack clinical relevant myocardial regeneration i.e. after myocardial infarction (MI), neonatal mammalian hearts possess the ability of complete cardiac recovery. We and others demonstrated complete cardiac regeneration in murine neonatal hearts after MI induced by experimental left ascending artery (LAD) ligation. Moreover, we reported the case of a neonatal human baby which completely recovered cardiac function following life-threatening perinatal MI.
However, the molecular mechanisms of mammalian neonatal cardiac regeneration are still unclear. Transcriptome analysis of regenerating neonatal mouse hearts established Igf1r as a potential candidate regulator for mammalian neonatal cardiac regeneration.
Here, we confirm the crucial role of Igf1r as an important factor for neonatal cardiac recovery after MI.
We induced cardiomyocyte-specific knock-down (KD) of Igf1r in postnatal day one (P0.5) mouse hearts using recombinant adeno-associated virus (rAAV9) delivered shRNAmirs and compared this group to a Renilla KD control virus group. Viral transduction efficiency was verified by immunohistochemistry (IHC) of rAAV9 delivered GFP expression. One day post infection both groups were randomized into a LAD surgery MI group or a SHAM control group. Cardiac function was assessed one day post injury (dpi) and 21 dpi. Three weeks post injury hearts were harvested and histologically analyzed.
One dpi hearts subjected to LAD surgery showed significantly reduced left ventricular ejection fraction (EF) compared to SHAM hearts in both viral groups by means of echocardiography. EF of SHAM hearts did not differ between viral groups. Whereas 21dpi LAD hearts in the Renilla control group functionally regenerated and were comparable to SHAM hearts, cardiac function of Igf1r KD hearts subjected to LAD ligation remained markedly impaired compared to all other experimental cohorts. In vivo functional depression in the Igf1r KD compared to the control groups was further supported by significantly increased fibrosis.
In summary, we confirm a critical role of cardiomyocyte specific Igf1r and its downstream signaling during neonatal cardiac regeneration. Thus, Igf1r may hold promise as a potential drug target to improve cardiac recovery in adult mammalian hearts.

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PhD programme
Molecular Cell Biology and Oncology (MCBO)

Non-protein-coding RNAs implicated in neurological diseases: from identification to function

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PhD programme
Gene Expression

Alzheimer's disease (AD) is characterized by progressive loss of memory and cognition. Here we demonstrate that non-coding RNAs play an important role in the pathology of AD. Brain cortex from AD patients was subjected to RNA-sequencing, which revealed the increased accumulation of tRNA-derived fragments (tRFs), as compared to healthy controls, which was subsequently verified by northern blotting. We demonstrate that the most prominent tRF candidates in AD are able to inhibit translation, concordant to their association with ribosomes, as shown by polysome profiling. Another class of ncRNAs, designated as small nucleolar RNAs (snoRNAs), is implicated in AD as well as in Prader-Willi Syndrome (PWS), a neurogenetic disorder caused by the deletion of imprinted genes on the paternally inherited human chromosome 15q11-q13. The deleted locus harbours the HBII-52 and HBII-85 repetitive clusters, with deletion of HBII-85 cluster causing PWS. Our work provides evidence that the mouse orthologues of the human brain-specific snoRNAs HBII-52 and HBII-85, can inhibit translation, potentially mediated via snoRNP formation,as demonstrated by electrophoretic mobility shift assay.

RNA cytosine methyltransferase NSUN3 regulates embryonic stem cell differentiation by promoting mitochondrial activity

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PhD programme
Gene Expression

First epigenetic modifications of both DNA and RNA were already described more than 60 years ago with the DNA modification, 5-methyl-2'-deoxycytidine (m5dC), as the best understood one to date. In contrast to DNA methylation, the study of RNA methylation was more challenging due to the lack of proper techniques. In case of 5-methylcytidine, the establishment of methods, such as bisulphite sequencing for RNA, made it possible to study RNA methylation at nucleotide resolution. m5C modifications in RNA can be installed by any of the seven proteins of the Nol1/Nop2/SUN domain (NSUN) family as well as the DNA methyltransferase family member DNMT2. Our previous data suggested that RNA methylation in mitochondria might be higher than in the cytoplasm. In a search for mitochondria-specific RNA cytosine methyltransferases (RCMTs), we targeted Nsun3, which was unstudied at that time and showed close sequence similarity to the mitochondrial enzyme Nsun4. Using CRISPR/Cas9 we introduced mutations in the presumptive catalytic domain of Nsun3 in embryonic stem cells (ESC) to study Nsun3's function. We show that Nsun3 methylates mitochondrial tRNAMet at position C34 in the anticodon loop. Nsun3 inactivation results in substantial impairment of mitochondrial translation and transcription and affects cellular glycolysis and respiration rates. Nsun3 mutant cells also exhibit compromised ESC differentiation. Together, these results suggest an important role for mitochondrial Nsun3 in ESCs even though these cells largely rely on glycolysis rather than oxidative phosphorylation for generating energy.

Poster # 59

Immunity, Infectious Diseases and Clinical Medicine

NMR solution structure and flexibility of the major apple allergen Mal d 1

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The major apple allergen Mal d 1 (Malus domestica) is the predominant cause of apple allergies in large parts of Europe and Northern America. Allergic reactions against this 17.5 kDa protein are the consequence of initial sensitization to the structurally homologous major allergen from birch pollen, Bet v 1. Consumption of apples can subsequently provoke immunologic cross-reactivity of Bet v 1-specific antibodies with Mal d 1 and trigger severe oral allergic syndroms, affecting more than 70% of all individuals that are sensitized to birch pollen.
To gain insight into this cross-reaction we characterized the structure, flexibility and functions of Mal d 1 using different NMR spectroscopic techniques.
Experimental structural data for Mal d 1 have not been available to date. We present the first NMR solution structure of this protein and show that Mal d 1 is highly flexible and can be stabilized by small hydrophilic compounds such as L-ascorbic acid. The protein fold is composed of a seven-stranded β-sheet and three α-helices, which is in accordance with the reported secondary structure of the major birch pollen allergen Bet v 1. This high structural similarity is the reason for the observed immunological cross-reactivity of the two major allergens from birch and apple.

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PhD programme
Chemistry

DC-iphering complement mediated HIV-1 incorporation and effects on DC function

Poster # 60

Immunity, Infectious Diseases and Clinical Medicine

The immune response to viral infections involves complex interplays between the virus and the immune system and targets elimination of the pathogen with minimum damage to the host. The complement pathway is spontaneously and vigorously activated after HIV-1 entry at mucosal surfaces, recognizing structures within the viral envelope. Complement fragments covalently bind to HIV-1, which makes attachment of HIV-1 to complement receptors (CR) on dendritic cells (DCs) more likely compared to interactions of rarely found non-opsonized virus with C-type lectins.
DCs are key modulators of immunity given their pivotal role in initiating and shaping adaptive immune responses against a vast variety of pathogens and cancer. HIV-1 has evolved strategies to evade DC-mediated antiviral immunity and to make matters worse the virus exploits DCs shuttles to promote its own dissemination. We recently showed that complement-opsonization of HIV-1 (HIV-C) allowed bypassing of restriction mechanisms in DCs and this was associated with and increased quality and quantity of virus-specific immune responses due to an enhanced DC infection and co-stimulatory activity. Therefore, we are interested in unraveling in detail how differentially opsonized HIV particles enter DCs, especially HIV-C, and how that affects signaling and antigen presentation. Now we are focus in breaking down the specific functions of CR3 and CR4 in more detail with respect to HIV-1 entry, processing and signaling in DCs to identify novel therapeutic host targets.
For that purpose we are generating stable knock-out cell lines using the CRISP-R Cas9 method and we are targeting complement receptors expressed on DCs, namely CR3 (CD11b/CD18) and CR4 (CD11c/CD18). To characterize the effects of the single receptors, we are generating CD11b and CD11c k.o. cells. To illustrate the effects of the common features of the receptors, we further created a CD18 k.o. cell line, which is also associated with loss of CD11a on myeloid cells. As model cell line we are using the THP-1 cells, a human leukemia monocytic cell line. The use of primary cells is not possible, because of the limited survival range in long protocols.

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PhD programme
Molecular Cell Biology and Oncology (MCBO)

Poster # 61

Immunity, Infectious Diseases and Clinical Medicine

Development of a HIV-Vaccine based on the VSV-GP vector

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PhD programme
Infectious Diseases

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 HIV infection.
We incorporated antigens from HIV or marker genes into the genome of VSV-GP and generated infectious viruses via reverse genetics. These viruses were analyzed in vitro for expression, infectivity, localization and conformation of the antigen. In mice distribution and kinetics of infected cells, antigen-specific and vector-specific immune responses were analyzed.
Infectious viruses containing antigens from HIV were generated. The addition of the additional antigen did not attenuate VSV-GP replication. HIV envelope variants were expressed in VSV-GP infected cells and incorporated into VSV-GP particles. Crucial epitopes for binding of broadly neutralizing antibodies against HIV such as MPER (membrane-proximal external region), CD4 binding side, V1V2 loop and V3 loop were present on the surface of VSV-GP-env particles. After intramuscular immunization of mice, 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. However, high HIV antibody titers were elicited in mice.
Taken together, VSV-GP is non-neurotoxic, induces potent immune responses, enables boosting and thus is a promising novel vaccine vector platform.

Establishment Of a 3D Respiratory Model to Study Fungal Infections

Poster # 62

Immunity, Infectious Diseases and Clinical Medicine

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PhD programme
Host Response in Opportunistic Infections (HOROS)

Aspergillus fumigatus has different clinical manifestations determined by the immune response of the host. In healthy individuals, fungal conidia are efficiently cleared by the immune system, while in immunocompromised hosts fungal persistence may result in invasive disease. To study interactions of A. fumigatus with upper respiratory tract epithelial and immune cells, we set up a perfused 3D human bronchial epithelial cell system prior adding primary myeloid dendritic cells, which comprise the dominant antigen-presenting cells in the respiratory mucosa. Growing of the normal human bronchial epithelial cells under air liquid interphase and perfusion illustrated a significantly accelerated and higher ciliogenesis, cilia movement, mucus-production and improved barrier function compared to growth under static conditions. Basolaterally adhered DCs migrated through the epithelium to the apical side in a time-dependent manner upon application of fungi.
We here illustrate, that in our in vitro three-dimensional respiratory tract system consisting of highly differentiated epithelial cells grown in ALI and under perfusion, primary DCs are fully functional and fulfill their tasks of sensing and sampling antigens present at the apical surface of epithelial cells.

Poster # 63

Immunity, Infectious Diseases and Clinical Medicine

Dorsolateral Nigral Hyperintensity on 3.0 Tesla Susceptibility-Weighted Imaging in Idiopathic Rapid Eye Movement Sleep Behaviour Disorder

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PhD programme
Clinical PhD

To assess loss of dorsolateral nigral hyperintensity (DNH) on high-field susceptibility-weighted imaging (SWI), a novel magnetic resonance imaging marker for Parkinson's disease (PD), in subjects with idiopathic REM sleep behaviour disorder (iRBD).
RBD is a parasomnia characterized by lack of muscular atonia during REM sleep associated with dream-enacting behaviour and unpleasant dreams. Prospective cohort studies of subjects with iRBD have shown that a majority go on to develop an alpha-synuclein-related neurodegenerative disease. These findings suggest that a proportion of subjects with iRBD could represent patients in a prodromal stage of PD. Recently, several groups have reported a novel MRI marker for PD based on the loss of DNH using iron-sensitive MRI including SWI at 3.0 and 7.0 Tesla (T).
15 iRBD patients were studied with 3T MRI. In order to compare DNH status (present or absent) between subjects with iRBD, healthy controls (HC) and PD we used imaging data of our recently published cohort of 104 PD subjects and 42 HC. DNH raters were kept blind to diagnostic categories and unilateral absence of DNH was classified as abnormal. The main per-protocol analysis to assess differences of DNH loss between groups was performed excluding scans of insufficient quality for reliable assessment.
Ten out of 13 (77%) iRBD patients showed loss of DNH, which was distinct from HC (corrected $p<0.001$) but similar to patients with PD (corrected $p=0.112$). Overall, one of 35 controls (3%) and 83 of 90 (92%) patients with PD patients showed loss of DNH.
In this study we found that 77% of iRBD patients showed loss of DNH, which is distinct from HC but similar to patients with PD. In PD this imaging finding has been linked to loss of dopaminergic cells in the Nigrosome 1 area of the substantia nigra (SN).This finding not only further supports the role of iRBD as a biomarker for prodromal PD but also raises the possibility that absent DNH on MR SWI might be a diagnostic tool to identify those iRBD subjects who harbour PD or other synucleinopathies affecting the SN.

Galactosaminogalactan secreted from *Aspergillus fumigatus* affects human platelet activity and stimulate complement system

Poster # 64

Immunity, Infectious Diseases and Clinical Medicine

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PhD programme
Host Response in Opportunistic Infections (HOROS)

Aspergillus (A.) and mucormycetes species cause severe infections in immunocompromised patients. To understand pathomechanisms and antifungal defence in more detail we studied the role of platelets and complement, which are important innate immunity elements. Recent own studies showed that the secreted fungal polysaccharide galactosaminogalactan (GAG) might be an important player since it affects platelet activity and activates the complement system.
Supernatant (SN) of Aspergillus and different mucormycetes isolates were collected after 2 days fungal growth and added to human platelets. GAG secretion, platelet activation and complement deposition on platelets were studied by scanning electron microscopy, FACS, confocal laser microscopy.
Incubation of platelets with A.fumigatus and A.flavus SN resulted in deposition of secreted fungal material on the platelet surface whereas no deposition was obvious when incubating the platelets with medium and SN of mucormycetes. This deposition of fungal material correlated with expression of GAG by A.fumigatus and A.flavus. Furthermore, the two SN triggered significant platelet activation. Other GAG effects on the platelets included the deposition of complement factor C3 and the formation of the C5b-9 complex on the platelet surface. A perfect correlation between presence of GAG and platelet activation/opsonization could be underlined by the comparison with different Aspergillus and mucormycete species. Furthermore, GAG-induced shedding of microparticles was noticed, which represent important pro-inflammatory mediators in the human body.
Our findings underline the hypothesis that GAG might represent an important fungal immunomodulatory molecule. Putative consequences of its activity include platelet-mediated antifungal attack and support of other elements of the immune network, but also thrombus formation and excessive inflammatory reactions.

Poster # 65

Immunity, Infectious Diseases and Clinical Medicine

Identification of amino acids that equip lipocalins with allergenic potential

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PhD programme
Gene Expression

The lipocalin family of proteins comprises most of the respiratory mammal allergens and some endogenous proteins. Previously, we used two pairs of homologous lipocalins each consisting of an allergenic and a non-allergenic lipocalin, to establish an in vitro system with dendritic cells (DC) and allogeneic T-cells able to distinguish between allergenic and non-allergenic immune responses. We could show that the interaction of lipocalins with DC can be decisive for the type of immune response induced.
Here we use this system to test a series of hybrid-lipocalins for their ability to induce TH1 or TH2 responses in order to identify the amino acids (AA) within lipocalins responsible for the induction of allergenic immune responses. Hybrid-lipocalins were designed by comparison of the AA sequences between allergenic and non-allergenic lipocalins to exclude any overlapping AA, and the subsequent pairing of allergenic Can f 1 and Fel d 4 to detect common "allergenic" AA. With this approach we found 11 "allergenic" AA, which were inserted into the non-allergenic Lcn-1 sequence. To further reduce the number of "allergenic" AA, we searched for additional common AA in the sequences of the cat allergen Fel d 7 and the horse allergen Equ c 1 as well as in the cow allergen Bos d 2 to finally reduce the "allergenic" AA to five and two, respectively. We produced all three hybrid-lipocalins with the Lcn-1 sequence as backbone replacing 11, 5 or 2 AA with the corresponding "allergenic" AA as recombinant proteins (HL11, HL5 and HL2). All three hybrid-lipocalins behaved as allergens in the above described system which allows the conclusion that the final 2 AA are responsible for priming DC to induce allergenic immune responses in co-cultures with allogeneic T cells. As a control we exchanged the 2 AA of HL2 in the Lcn-1 sequence on a nearby position. This control-lipocalin was not allergenic. We conclude that not only the identity but also the position of the AA in the lipocalins accounts for their allergenic potential.
Further investigations are needed to identify the mechanism(s) employed by lipocalin allergens to prime DC for the induction of allergenic immune responses.

Molecular properties of breast cancer cells with increased migratory capacities

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PhD programme
Molecular Cell Biology and Oncology (MCBO)

Despite the development of novel diagnostic and therapeutic approaches, approximately 20-30% of patients with early breast cancer (BC) develop distant metastasis, which affect the 5-year survival rate. However, BC patients with distant metastasis demonstrate different treatment response that can be associated with specific molecular patterns of metastatic cells. Hence, investigation of the molecular properties of cells with increased metastatic potential is required to better understand how to improve therapy response in BC patients with distant metastases.
Three carcinoma cell lines, MDA-MB-231, T47D and Au565, representing the most diffused molecular subtypes of breast cancer (triple negative, luminal A and HER2-positive BC) were used to obtain cells with increased migratory abilities by using the Boyden chamber. Newly received migratory BC cells were compared with their parental counterparts for their physiological and molecular properties, by combining cell viability and clonogenic assays after the application of either ionizing radiation or chemical compounds. Adhesion assay, scanning electron microscopy, FACS analysis and Western Blot were also used in the study.
BC cells with increased migratory abilities demonstrated different sensitivities to ionizing radiation or chemical agents compared to their parental counterparts. All migratory cell lines were enriched for the content of tumor initiating cells (TICs) carrying the surface markers CD44+/CD24-/ALDHA1. Additionally, BC migratory cells showed enhanced adhesive capacities and activation of epithelial-to-mesenchymal transition. In parallel, BC migratory cells were characterized by affected cell cycle regulation that markedly contributed to their proliferation capacities and sensitivity to irradiation and chemotherapeutics.
BC cells with increased migratory abilities demonstrate their aggressive behaviour through dysregulation of cell cycle progression, changed membrane properties and activation of pro-survival mechanisms associated with affected therapy response. This observations seem, therefore, simulate the different sensitivities to the applied treatment approaches in metastatic BC patients.

Poster # 67

Immunity, Infectious Diseases and Clinical Medicine

Identification of genetic variants and phenotypic correlates of the complement activation system in the Cooperative Health Research in South Tyrol (CHRIS) study

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PhD programme
Host Response in Opportunistic Infections (HOROS)

The complement system is a fundamental component of the innate immune response. It can be activated via classical, lectin and alternative pathways, converging in the generation of C3 convertases and finally leading to the formation of the terminal complement complex (TCC). To enhance the function of antibodies and phagocytic cells to remove pathogens from an organism, complement activation has to be controlled by different regulators. The association of complement protein deficiencies with various diseases has been already investigated, like factor H deficiency and occurrence of chronic renal diseases or age-related macular degeneration. The aim of this study is to identify genetic polymorphisms (SNPs) associated with elevated or decreased activation capability of the complement system via all three pathways. We assessed the functional activity of the three pathways in addition to the terminal pathway by using a commercially available assay, the WIESLAB complement system screen on serum samples from 5000 participants of the Cooperative Health Research In South Tyrol (CHRIS) study. These subjects were already genotyped on one million SNPs. Genome-wide association analyses were performed on the three pathways, using linear mixed models to account for family structure and relatedness in general. Identified SNPs will be tested for association with cardiovascular, kidney, autoimmune diseases, as well as neurological conditions. The presence of samples with a low functional activity (< 10%) has been investigated. Given that the high lipemic index could lead to that condition, sensitivity analyses will be performed to understand the specific role of lipemia on the association between previous identified SNPs and the three activation pathways. Those results will allow to better understand the genetic role of the complement system activation.

Poster # 68

Immunity, Infectious Diseases and Clinical Medicine

Orphan receptor NR2F6 in germinal center reaction - implication for autoimmunity

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PhD programme
Molecular Cell Biology and Oncology (MCBO)

The germinal center reaction (GC) is the site of B cell affinity maturation and memory formation. As such the GC is critical for humoral immunity and vaccine responses. Follicular CD4+ helper T (Tfh) cells are important for the formation and maintenance of the GC reaction through several mechanisms including, cytokine production and co-receptor stimulation, and the subsequent recruitment of follicular B cells to the GC. Because of their importance in GC regulation Tfh cells must be tightly regulated as altered Tfh activity can lead to pathogenic immune responses and auto-immunity. We have previously demonstrated the role for NR2F6 as a check on CD4 Th17 driven auto-immunity and CD4 Th1 and CD8 T cell cancer immune surveillance. In humans Nr2f6 expression in CD19+ B cells derived from systemic lupus erythematosus patients is significantly reduced. Therefore we wanted to investigate the role of NR2F6 in GC derived auto-immunity.
Loss of Nr2f6 leads to a dramatic increase in GC reaction as both Tfh cell and GC B cell numbers are enhanced during the immune response to sheep red blood cells (SRBC) in vivo. We demonstrate a significant role for NR2F6 within the CD4 T cell compartment by using adoptive cell transfer of a small number of CD4 OT-II Nr2f6 deficient T cells into wild-type hosts we show is sufficient to replicate the GC reaction in globally Nr2f6 deficient mice after OVA-alum immunization. In vitro NR2F6 acts as a suppressor of the critical Tfh cytokines interleukin (IL)-21 and IL-4.
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 after NP-OVA immunization. Investigate B cell affinity maturation and long term plasma cell formation. Determine which cytokines are responsible for the increased GC response in Nr2f6 deficient mice.

Poster # 69

Immunity, Infectious Diseases and Clinical Medicine

The role of Natural killer cells in early UVB-induced carcinogenesis

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Post-Doc

Immunosurveillance of tissue is an important mechanism by which the immune system prevents cancer development. In an earlier study with chemical carcinogenesis we demonstrated that Natural Killer (NK) cells and Langerhans cells (LC) are necessary for the efficient elimination of DNA-damaged keratinocytes introduced by the application of the carcinogen 7,12-Dimethylbenzo(a)anthracen (DMBA). Mechanistically TNF-α, mostly produced by LC, was responsible for the induction of the chemokines CCL2 and CXCL10 which in turn mediated the recruitment of NK cells to the epidermis. The aim of this project is to gain new insights into the role of NK cells in the immunosurveillance of UV-induced skin cancer. UV-irradiation of skin is known to induce DNA damage in skin cells which can accumulate and lead to skin cancer. Our results show increased TNF-α levels shortly after irradiation and an upregulation of the chemokines CCL2 and CXCL10. When we depleted NK cells in C57BL/6 mice by injecting an anti-NK1.1 antibody (PK136 clone) before UV-irradiation, significantly more DNA-damaged keratinocytes were present in skin sections indicative of an accumulation of DNA-damaged cells. These results suggest that cells from the innate immune system act as early mediators in the immunosurveillance of UVB -induced carcinogenesis in the skin.

Ferritin: A link between macrophages and stress erythropoiesis

Poster # 70

Immunity, Infectious Diseases and Clinical Medicine

Erythrocytes are needed for oxygen delivery to all sites of the body. Every second more than two million new erythrocytes are released from the bone marrow of human adults, highlighting the tremendous turn-over and need of organized replenishment of these cells. In the bone marrow, being the major site for the maturation and production of red blood cells, 'erythroblastic islands' (EBIs) are formed within an extravascular niche. Such EBIs are composed of up to 30 erythroid progenitor cells, which interact with a central macrophage. Erythroid differentiation goes along with hemoglobin synthesis, which requires large amounts of iron. Up to date it remains unanswered, whether central macrophages in the bone marrow can directly donate iron to developing erythrocytes, to meet the tremendous amounts of iron needed for the hemoglobinisation.
Using a myeloid-specific ferritin H knockout strain, we examine the connection between central macrophages, erythropoiesis and iron metabolism with special focus on the role of ferritin in the context of stress erythropoiesis.
Upon weaning, female FtHWt (FtHflox+/+ LysMCre-/-) and FtHcKO (FtHflox+/+ LysMCre+/+) mice being on a C57BL/6 background, were fed an iron adequate diet (20mg/kg iron) for 8 weeks. Consequently stress erythropoiesis was induced via withdrawal of blood from the V.facialis on three consecutive days. On day 4 the bone marrow, spleen and blood were collected for FACS analysis.
At steady state FtHcKO mice did not show any abnormalities in red blood cell count, hematocrit or hemoglobin level when compared to FtHWt mice. However, during stress-induced anemia, which causes extramedullary erythropoiesis, we observed a significantly increased mortality rate in the myeloid-specific mutant mice. This observation was paralleled by lower spleen weight, higher amounts of reticulocytes and abnormalities in complete blood count among FtHcKO animals that survived. These results indicate that macrophage-specific ferritin plays a role in erythropoiesis, either directly or indirectly. Further investigations will address the mechanism, why FtHcKO mice are more susceptible to death after bleeding, to uncover the contribution of ferritin for erythropoiesis.

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PhD programme Host Response in Opportunistic Infections (HOROS)

Early markers of an increased cardiovascular risk in former preterm Tyrolean preschoolers

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PhD programme
Clinical PhD

Cardiovascular disease is the leading cause of death worldwide. A growing body of evidence suggests that prematurity is associated with an unfavorable cardiovascular risk profile in adult life. Whether alterations already exist at an earlier age is currently unknown, but timely detection is crucial for effective disease prevention. The aim of the present study was to assess the presence of traditional as well as novel indicators of an increased cardiovascular risk in former very preterm infants at a preschool age.
Former preterm infants born between 01/01/2007 and 07/31/2009 with a gestational age <32 weeks at birth were followed-up at 5-7 years of age. Healthy same-aged children born at term served as controls. Examinations at study visit included collection of anthropometric data, blood pressure measurements, fasting blood sampling (glucose homeostasis, lipid status, plasma amino acid profiles, thyroid function) and echocardiographic assessments (aortic intima-media thickness, elastic properties).
89 former term and 93 former preterm infants were included in the study. In comparison to children born at term, former preterm infants had higher systolic and diastolic blood pressure readings, fasting glucose concentrations, homeostasis model assessment index, and cholesterol levels. Systolic prehypertension and hypercholesterolemia were significantly more prevalent in the preterm group. In addition, former preterm infants displayed changes in plasma amino acid profiles and thyroid hormone levels consistent with an unfavorable cardiovascular risk profile. Aortic intima-media thickness did not differ between groups, but in the descending abdominal aorta, significantly altered elastic properties (decreased distensibility, increased stiffness index β) were detected in the preterm group. All observations were independent of major covariates such as age, sex, childhood nutrition profiles and current body-mass index z-score.
Former preterm infants show an adverse cardiovascular risk profile already at a preschool age. Implementation of routine cardiovascular follow-up programs for these children might be warranted.

Prothrombin complex concentrate in patients with bleeding complications related to rivaroxaban

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PhD programme
Infectious Diseases

Non-vitamin K antagonist oral anticoagulants (NOACs) pose a great challenge for physicians in life-threatening bleeding events. The aim of this study was to test the efficacy of the reversal of the NOAC rivaroxaban with PCC (prothrombin complex concentrate), a non-specific reversing agent.
Patients with life-threatening bleeding events during rivaroxaban treatment were included and administered 25 U/kg of PCC. Blood samples were collected immediately prior to as well as after PCC treatment at predefined time intervals. The primary endpoint was defined as the difference in thrombin generation (TG) prior to and ten minutes subsequent to PCC treatment.
Fourteen patients, of whom the majority suffered from intra-cranial haemorrhage (ICH) or subdural haemorrhage (SDH), were included and administered PCC. The results show that the area under the curve (AUC, TG) significantly (p=0.001) improved by 68% and the peak thrombin generation (Cmax, TG) by 54% (p=0.001) during PCC treatment.
In addition, the quick value (prothrombin time: quickPT) significantly improved by 28% and the activated partial thromboplastin time (aPTT) was decreased by 7% ten minutes after PCC administration. Cmax was reduced at baseline, but not AUC, aPTT or quickPT. Lag time until initiation (TG, tlag), thromboelastometry clotting time (CTEXTEM) and time to peak (TG, tmax) correlated best with measured rivaroxaban levels and were out of normal ranges at baseline, but did not improve after PCC administration. In 77% of the patients the bleeding (ICH/SDH-progression) ceased following PCC administration. During the study three participants passed away due to other complications not related to the treatment with PCC. The possibility of thrombosis formation was also evaluated 7 days after administering PCC and no thromboses were found.
The study has shown that the use of PCC improved AUC, Cmax, quickPT and aPTT. However, of these parameters, only Cmax was reduced at the defined baseline. It can be concluded that CTEXTEM, tlag and tmax correlated best with the measured rivaroxaban levels. The study drug was found to be safe; nonetheless, additional studies specifically targeting the assessment of clinical endpoints should be performed to further confirm these findings.

Poster # 73

Immunity, Infectious Diseases and Clinical Medicine

Cardiovascular health in Tyrolean Adolescents - EVA Study

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Atherosclerosis is the most common cause of cardiovascular disease - the leading cause of mortality. Recent findings suggest that vascular changes start early in life and are associated with common risk factors like smoking, obesity, and hypertension. Therefore, this study aims to assess vascular health and predictors for early vascular ageing in Tyrolean adolescents and to promote healthy lifestyle in this population.
Volunteering Tyrolean schools and companies have been invited to take part in this study. Standard vascular risk factors like obesity, blood pressure, total cholesterol, fasting glucoseare assessed with a special focus onlifestyle factors like smoking, physical activity, and diet.Vascular changes are evaluated by measuring carotid intima-media-thickness of the common carotid artery with high resolution ultrasound. Metrics of vascular health in youngsters were defined according to the guidelines of the American Heart Association.
1264 adolescents with a mean age of 16 years are enrolled, of which 53.9% are female.30.6% were current or former smokers.The frequency of health metrics in the ideal range were 9.9% for diet, 42.7% for physical activity (≥ 60 minutes per day), 70.0% for blood pressure (< 90th percentile), 70.0% for total cholesterol (<170 mg/dl) and 81.1% for BMI (< 85th percentile). We found differences between boys and girls regarding ideal cardiovascular health. Girls more often reported an ideal diet (11.9% vs 7.5%, p=0.009), while boys had better results regarding smoking behavior (74.2% vs 65.2%, p=0.01), physical activity (56.1% vs 33.1%, p=0.000), and total cholesterol (83.0% vs 59.6%, p=0.000).
Risk factors for atherosclerosis are frequent in Tyrolean adolescents and cardiovascular health in the young must be improved by promotion of a healthy lifestyle.
Further analysis is needed to find out whether there is an association of risk factors and vascular changes already in this age group.

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PhD programme
Clinical PhD

DCs exposed to opsonized HIV-1 are not able to restore CD8+ T cell functions during bacterial co-infection

Poster # 74

Immunity, Infectious Diseases and Clinical Medicine

HIV spontaneously activates complement (C) in semen and at mucosal surfaces and is therefore opsonized with a cloud of covalently bound C3 products in vivo. We earlier illustrated that C opsonization of HIV-1 and HIV-2 significantly modulates dendritic cell (DC) function and antigen-presenting capacity. Upon bacterial co-infection at mucosal surfaces pathogenic bacteria and their microbial products activate DCs and thereby might alter their function and T cell-stimulatory capacity regarding HIV-1, which we investigated during this study. We found similar binding of non- (HIV) and complement-opsonized HIV (HIV-C) to immature DCs (iDCs) and LPS-matured DCs (LPS-DCs), while internalization of both, HIV and HIV-C, was significantly higher in LPS-DCs. About one third of viral particles were detected in the cytoplasm independent on the opsonization pattern in iDCs, while in LPS-DCs solely endocytosis – but not fusion - was observed. HIV-C was recently shown by our group to overcome restriction in DCs in contrast to HIV. This was not the case for LPS-DCs, which were not productively infected by HIV-C. Transfer of HIV from iDCs and LPS-DCs to CD4+ T cells was similar and significantly higher than HIV-C transfer, pointing to an antiviral effect of DCs loaded with HIV-C independent on co-infection. In contrast we found a weaker capacity of HIV-C-LPS-DCs to expand and activate CD8+ T cells compared to HIV-C-DCs. These results indicate an impact of co-infection on the CTL-stimulatory capacity of DCs in presence of pathogenic gram-negative bacteria during acute infection. Our ongoing studies investigate the influence of Chlamydia spp, the main cause of non-gonococcal urethritis, on dendritic cell function to better reflect the physiological conditions occurring during bacterial co-infections.

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PhD programme
Molecular Cell Biology and Oncology (MCBO)

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

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PhD programme
Molecular Oncology

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. With standard treatment the 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 pseudotyped with LCMV glycoprotein (VSV-GP) is a particularly promising candidate due to its fast mode of action, high titer production, absence of pre-existing immunity and broad tumor tropism.

Here, we propose the use of oncolytic VSV-GP for the treatment of lung cancer.

VSV-GP was found to efficiently infect and lyse most of the cell-lines in vitro. However, analysis of the innate immune response of lung cancer cells to VSV-GP revealed sensitivity to type I Interferons and induction of an antiviral state of the cells. Weakening the antiviral response by knocking out interferon receptor 1 (IFNAR1) on mouse lung cancer cells resulted in significantly enhanced oncolytic activity of VSV- GP. In vivo, however, the effect of IFNAR1 knockout in LLC-1 tumors had little effect on the treatment response in a subcutaneous syngeneic model. In contrast, xenogtraft tumor models of human lung cancer showed complete remission after either intratumoral or systemic treatment with VSV-GP.

To visualize virus activity in vivo, xenograft and syngeneic s.c. tumors were analyzed for bioluminescence activity after intratumoral or systemic treatment of luciferase-expressing VSV-GP. Virus replication was restricted to tumor tissue only and was recorded for several days post infection. Importantly, studies on syngeneic models with bilateral tumors revealed successful tumor-to-tumor spread of virus after unilateral injection in immune-competent hosts.

Ongoing studies are currently addressing the interaction of the triad VSV-GP – immune system – tumor to identify resistance factors and improve therapeutic outcome of 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.

Combination therapies to improve dendritic cell-based treatment of melanoma

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PhD programme
Molecular Oncology

Dendritic cells are essential mediators of immune responses due to their ability to capture and present antigens to T cells. In anti-tumour responses however, their function in the tumor microenvironment is compromised due to antigen inaccessibility, lack of proper activation signals and immunosuppression by the tumor. Thus, successful immunotherapy must overcome these hurdles by combining tumor-targeted approaches and immunotherapy.

Our aim is the induction of anti-tumor immunity by combining tumor-targeted therapy with DC-based immunotherapy and checkpoint blockade inhibitors.

Tumor cell death will be induced by inhibitors of the BRAFV600E kinase or the glutamate pathway in different melanoma mouse models. These approaches will be tested in a spontaneous melanoma model (tg(Grm1) EPv mice) driven by the overexpression of the metabotropic glutamate receptor 1 and tamoxifen-inducible melanoma in Tyr::CreER-CreBRAFV600E/wtPten-/- mice. Additionally, transplantable melanoma mouse models will be used, B16.OVA, and BRAFV600E-expressing cell lines, SM1WT1 and D4M. The expression of selected melanoma antigens will be tested in each model.

Melanoma antigens will be delivered to skin DC by antibody-antigen fusion proteins or liposomes for presentation to CD8+ T cells by different means (intradermal vaccination compared with epicutaneous immunization on laser-porated and tape-stripped skin). Furthermore, checkpoint inhibitors will be added (anti-PD-1, anti-PD-L1).

The expression of three melanoma-associated proteins (gp100, trp-2 and MAGE-A2) was examined by RT-PCR and confirmed in B16-derived tumors and melanoma lesions from tg(Grm1)EPv mice. The SM1WT1 tumours had lost melanoma antigens. The D4M tumours will be tested as well. These melanoma antigens will be used for the production of antibody-antigen fusion proteins delivering the antigens specifically to either DEC-205- or Langerin- expressing skin DC subsets.

The efficacy of each immunization method for the delivery of antibody-antigen fusion proteins and in the induction of skin DC activation and T cell responses will be compared to standard intradermal injection.

Tumor growth arrest and cell death induction by BRAFV600E kinase and glutamate pathway inhibitors will be assessed and combined with DC-based vaccination using antibody-antigen fusion proteins. The most prominent combination will be combined with checkpoint blockade inhibitors in order to further boost the anti-tumour immune response.

Poster # 77

Immunity, Infectious Diseases and Clinical Medicine

An efficient first line immune defense is initiated by Aspergillus fumigatus devoid of β-1,3-glucan

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PhD programme
Infectious Disease

In this study, 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.
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.
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.
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 production of pro-inflammatory cytokines, thereby resulting in a favorable TH1/TH17 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.

Laser-assisted skin immunization to target dendritic cells in human

Poster # 78

Immunity, Infectious Diseases and Clinical Medicine

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Post-Doc

Skin dendritic cells (DC) are antigen presenting immune cells which induce immune responses against cutaneous infection and tumours. Due to their localization in the skin, they are able to recognize cancer cells developing in the skin and to start an immune response against tumours. The immunotherapeutic approach called epicutaneous immunization aims at loading DC subtypes directly with tumour antigens. To improve epicutaneous immunization we intend to load skin DC with antibody-antigen (Ab-Ag) conjugates against DC surface molecules, such as the lectin receptors DEC-205 and Langerin that are essential for antigen incorporation. An essential improvement of epicutaneous immunization is expected from a laser poration of the skin. An infrared laser (P.L.E.A.S.E.® Laser System, Pantec Biosolutions) creates micropores in the skin by excitation of water molecules. These micropores should allow macromolecules to diffuse into the skin, and therefore enable the transcutaneous application of molecules with high molecular weight, like antibodies. Through these pores it will be possible to deliver larger molecules such as Ab-Ag vaccines together with adjuvants for immunization. Human and murine skin samples were prepared to determine the optimal parameters for ablation of epidermis and dermis. The laser-induced thermal damage was investigated. DC targeting by antibodies against Langerin and DEC-205 was evaluated. We were able to induce pores of definable depths and no increased apoptotic signals were found in the surrounding of the pores. However, the DC targeting efficiency after intradermal injection was found to be more effective than the new laser treatment. Future experiments will investigate the benefit of co-applied adjuvants and the immune-stimulatory capacity of antigen-targeted DC in laser treated human and murine skin.

Poster # 79

Immunity, Infectious Diseases and Clinical Medicine

Effects of systemic iron perturbations on mitochondrial fitness and on cellular metabolism in vivo

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PhD programme
Host Response in Opportunistic Infections (HOROS)

Mitochondria are dynamic organelles, that play a role in a variety of cellular functions, ranging from cellular death to ATP production. Among these, mitochondria are also involved in maintaining iron homeostasis, an essential co-factor of the oxidative phosphorylation, and of the cellular energy metabolism. Thus, imbalances of iron homeostasis could impact not only on the mitochondrial fitness but also the cellular metabolic processes. Nevertheless, little is known on that. Therefore, we aimed at investigating the effects of systemic iron perturbations on the mitochondrial function, and on the peripheral blood metabolites.
Mitochondrial respiration was studied in fresh liver samples of 10-week old C57BL/6N mice, receiving either normal- or high iron (5 g/kg)-diet for up to three weeks before being sacrificed. Livers were homogenized and mitochondrial respiration was assessed by means of high resolution respirometry (OROBOROS Instruments, Austria). Peripheral blood was sampled overtime, and metabolomics analysis was performed by using liquid chromatography-mass spectrometry (LC-MS).
Our ongoing experiments indicate that dietary iron supplementation affects the phosphorylation system in the mouse liver of C57BL/6N mice. The analysis of peripheral blood metabolites suggests that the iron diet is driving to metabolic changes especially in the urea cycle and in the insulin signaling.

Exploring the role of USP27x as a regulator of the BH3-only protein Bim

Poster # 80

Immunity, Infectious Diseases and Clinical Medicine

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Master-Student

The BH3 only protein BIM, a member of the Bcl-2 Family, is an important initiator of the intrinsic apoptosis pathway and plays a major role in normal tissue homeostasis and disease. BIM levels and activity are regulated at multiple levels. One critical pathways regulating BIM protein stability is the oncogenic RAS-RAF-ERK signaling module, which leads to the phosphorylation of one of the three isoforms of BIM, BIM-EL, on Serine-69 and thereby promotes its ubiquitination and proteosomal degradation. USP27x is the only known DUB that is able to remove ubiquitin-chains from phosphorylated BIM-EL. This is important as it leads to the stabilization of BIM and consequently sensitization of different cell types to apoptosis.
We analyzed the effect of USP27x deficiency on BIM levels and its consequences on cell death in in-vivo as well as in-vitro models. Using bone marrow form Usp27x-deficient animals, we observed reduced BIM-EL levels in neutrophil-progenitor lines generated upon transduction with HoxB8-encoding retro-virus. Furthermore, these cells were less susceptible to cell death while primary lymphocytes and myeloid cells lacking USP27x showed normal cell death susceptibility. Interestingly, the interaction between USP27x and BIM may have an effect on another member of the Bcl-2 Family, namely the pro-survival protein MCL-1 which was also reduced. Finally, no differences were observed between WT and USP27x-deficient mice in the context of Eμ-Myc driven lymphomagenesis, known to be suppressed by BIM, contrasting reports that USP27x might serve as an oncogene.

Poster # 81

Pharmacology and Neuroscience

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

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Special AT-rich sequence binding protein 2 (SATB2) is a risk locus for schizophrenia and encodes a highly conserved DNA-binding protein that regulates higher order chromatin configuration. We were able to show that Satb2 expression in the adult mouse forebrain is necessary for synaptic plasticity and long-term memory formation.
In order to explore the molecular mechanisms by which Satb2 contributes to memory formation and cognition we performed a proteomic analysis of Satb2-interactors. We demonstrate that Satb2 forms complexes with proteins of the nuclear lamina that tether chromatin to the nuclear envelope. Lamina-associated proteins are known regulators of gene transcription and nuclear shape. In this current study we investigated the role of Satb2 in nuclear morphology of pyramidal neurons.
By gain- and loss-of-function assays we show that Satb2 regulates the nuclear morphology of hippocampal neurons in vitro. In vivo the number of nuclear infoldings is greatly reduced in the hippocampus and the cortex of Satb2 conditional knock-out mice. Nuclear dysmorphology is rescued together with long-term memory deficits when Satb2 is re-expressed in the hippocampus of Satb2 conditional mutants.
Based on our findings we hypothesize that Satb2 is part of an inner nuclear membrane protein complex that is involved in plasticity-induced changes in nuclear geometry. Consequent changes in nuclear morphology and chromatin organization might be important for gene expression underlying the formation of long-term memory.

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PhD programme
Signal Processing in Neurons (SPIN)

Temporal lobe epilepsy and dynorphin: development of a gene therapy

Poster # 82

Pharmacology and Neuroscience

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.

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PhD programme
Signal Processing in Neurons (SPIN)

Plasticity of the presynaptic wiring of orexin-containing neurons in basal conditions

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PhD programme
Neuroscience, Psychological and Psychiatric Sciences

The orexin (OX)/hypocretin peptides are released by neurons located in the lateral hypothalamus. Orexinergic neurons distribute fibers to many regions of the neuraxis, and are implicated in multiple physiological functions including energy homeostasis, arousal and sleep-wake stability, motivated behaviors. Previous studies have shown that the activity of orexinergic neurons varies in relation to the animal's behavioral state. The present investigation tested whether such daily variation of neuronal firing could be subserved by synaptic plasticity phenomena in basal conditions. The experiments were conducted following ethically approved protocols. Mice were sacrificed during day or night, at times when sleep or wake predominance, respectively, were assessed by electroencephalography in matched mice. Triple immunofluorescence in epifluorescence and in confocal microscopy, as well as quantitative analyses pursued in pairs of sections, were used for the evaluation of glutamatergic and GABAergic contacts on cell bodies containing OX-A. Presynaptic markers included the vesicular glutamate transporter (VGluT)2 or the vesicular GABA transporter (VGAT) together with synaptophysin. Synapse scaffold proteins (the postsynaptic density protein 95+ for excitatory contacts, and gephyrin for inhibitory contacts) were used as postsynaptic markers. The results indicated that the combined total number of glutamatergic and GABAergic varicosities apposed to OX-A cell bodies was similar during day and night. However, glutamatergic varicosities were significantly more numerous at night, whereas GABAergic varicosities prevailed significantly at daytime. The study with postsynaptic markers confirmed the daytime prevalence of excitatory contacts, and nighttime prevalence of inhibitory contacts, indicating also that they formed synapses on OX-A neurons. The findings thus point out a striking daily oscillation in the axosomatic wiring of orexinergic neurons, with a switch from a prevalence of excitatory innervation during wakefulness to a prevalence of inhibitory innervation during sleep. This daily reorganization could represent a key mechanism of plasticity of the orexinergic network which links bodily functions, alternation of vigilance states and environmental cues. Studies are currently ongoing to verify the occurrence of such plasticity mechanisms of orexinergic neurons in aged mice and in murine models of Alzheimer's disease.

Involvement of the Olfactory System in neurodegeneration

Olfactory pathway is widely affected in neuordegenerative diseases. In particular, each specific phatogenic protein namely amyloid-beta and TAU, alpha-synuclein, TDP-43 and PrP are aggregated under amyloid plaques in olfacctory bulb. This study is focuses on the involvment of the olfactory neurons in the molecular mechanisms of neurodegeneration process. In particular, abnormal post-translational modificcation of these proteins may lead to misfolding, aggregation with toxic effect on cell-metabolism. Based on previous findings, we suggest that the initial molecular mechanisms take place in the olfactory neurons and invastigating this olfaction micrp-environment we might obtain the insights on the very early neuronal pathological change leading to neurodegeneration.
We carried out an immunocytochemistry and biochemical studies on olfactory mucosa obtained from 20 healty subjects. Each subjects underwent to a nasal swab and cells were collected and processed for the immunostaining with speciific antibodies to Olfactory Marker Protein (OMP, [FL-163], SantaCruzBiotech), TAU ([MAB631], Millipore), alpha-synuclein ([4D6], ABCAM), prion protein (PrP, [6D11], Covance), TDP-43 (10782-2-AP, Proteintech) and amyloid-beta ([6E10], Covance). Post-translational modifications, including phosporylation, glycosylation, ubiquitination were tested by immunoblot analysis.
In all analyzedd samples immunocytochemistry showed a consistent cell population positive to OMP. In particular, we observed diffuse cytosolic positivity to OMP in cillited cells, likely mature olfactory neurons. Positive staininig to NeuN and Tuj-1 were also observed. Stem cells, with rounded and swollen cell-bodies were positive for Nestin. Amyloid-beta was localized at the plasma membrane like alpha-synuclein. TAU and PrP dysplayed a cytosolic diffusion, while TDP-43 a peri-nuclear localization.
This study shows for the first time the cell distribution of amyloid-beta, alpha-synuclein, TAU, TDP-43 and PrP, involved in neurodegeneration in the olfactory neurons. We also showed that these proteins physiologically undergo to an abberant processing at the basis of the cell-damage.

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PhD programme
Neuroscience, Psychological and Psychiatric Sciences

Poster # 85

Pharmacology and Neuroscience

Is NgR- signaling required for the maintenance of the structural integrity of mouse fungiform and circumvallate taste buds?

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PhD programme
Signal Processing in Neurons (SPIN)

Gustation is mediated by taste buds, which are housed in specialized taste papillae found in a stereotyped pattern on the surface of the tongue. While fibers of the facial and glossopharyngeal nerves innervating the taste bud convey taste sensation, fibers of the trigeminal nerve project to the perigemmal epidermis of the taste papillae and account for somatosensation. The molecular mechanisms responsible for the development and maintenance of taste buds are incompletely understood, but chemosensory innervation is critical for the maintenance of taste buds throughout the lifespan. We recently discovered that the Nogo66 receptors (NgRs), which restrict axonal arborization are expressed by geniculate and trigeminal neurons during the period of chemosensory innervation, but it is not known whether gustatory chemosensory neurons require NgR-signaling for innervation or phenotypic maintenance of taste buds. We are currently examining to what extent NgR-signaling is necessary for chemosensory innervation and maintenance of taste buds, and whether NgRs have an early function in the formation of taste papillae. These experiments may aid in the rational design of therapeutic interventions for diseases and injuries of peripheral sensory neurons, potentially including abnormal or impaired taste sensation, conditions known as dysgeusias and hypogeusias.
Preliminary results reveal that tongues from NgR1/2- null mutant mice are similar in the overall topography to those from wild-type littermates. Counting of fungiform papillae on surface-stained tongues from three wild-type and four null mutant mice at P16–P17 showed no difference in the total number of papillae. However, loss of NgR1/2 significantly affected taste bud volume of fungiform papillae by 50%. Further studies are performed to investigate whether the reduced taste bud size correlates with changes in gustatory innervation and/or changes in the distribution of taste cells.
Our preliminary data suggest that postnatal NgR 1/2 expression may serve as an important factor for taste bud development and morphology.

Nuclear lamina-chromatin interactions in neuronal cells

Poster # 86

Pharmacology and Neuroscience

Special AT-rich sequence binding protein 2 (SATB2) is a risk locus for schizophrenia and encodes a highly conserved DNA-binding protein that regulates higher order chromatin configuration. We were able to show that deletion of Satb2 from adult mouse forebrain impairs long-term memory formation (Jaitner et al., 2016).
We describe here that Satb2 regulates nuclear morphology both in vitro and in vivo and causes the formation of nuclear infoldings (NIFs). In addition we found that Satb2 interacts with proteins of the inner nuclear membrane (INM) Lem2, Lem3, Baf and Lap2. Complexes of these lamina-associated proteins are known regulators of both gene transcription and nuclear shape.
To test whether the interaction between Satb2 and inner nuclear membrane proteins is required for nuclear infoldings, we used an RNAi approach to reduce Lem2 protein level. The experiment confirmed that Satb2-induced changes in neuronal nuclei geometry depends on Lem2 interaction.
To define protein domains in Satb2 that are necessary for Satb2–INM proteins interaction and consequently for modulation of NIFs in hippocampal neurons we generated deletion constructs of Satb2. These deletion mutants were tested in co-immunoprecipitation experiments. Interactions with candidate proteins (Lem2, Baf1) were analyzed and compared to the full-length protein. Our results reveal that the DNA binding domains CUT-like in the N-terminal half of the protein, and the two CUT domains in the C-terminal half are the domains responsible for Satb2 interaction with the INMs proteins. Our preliminary data show that all these three domains (CUT-like, CUT1 and CUT2) are necessary for the formation of NIFs, suggesting that in order to change the nuclear morphology Satb2 needs not only the binding to the nuclear envelope but also the DNA binding.
Based on our results we hypothesize that Satb2 modulates association of specific chromatin loops with the inner nuclear membrane and consequently regulates nuclear shape and genes expression underlying the consolidation of memory.

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PhD programme
Signal Processing in Neurons (SPIN)

Poster # 87

Pharmacology and Neuroscience

Different neuronal activation patterns in different amygdala nuclei after fasting and fear extinction

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PhD programme
Neuroscience

Anxiety disorders are the most frequent brain disorders imposing a significant burden to affected individuals, their families and the whole society. Dys-regulation of fear, anxiety and related behavioral disturbances are hallmarks of anxiety disorders. On the other hand, eating disorders are emotional disorders linked to anxiety and depression. However, how feeding affects cognitive skills and anxiety- or fear-related processes is not known. To investigate this interaction and the underlying neuronal circuitries is the focus of this project.

Fear was investigated by Pavlovian fear conditioning, in which an initially neutral stimulus, such as a tone (CS), is repetitively paired with an unconditioned stimulus (foot shock, US). The resulting fear memory is characterized by increased freezing behavior to the CS. Importantly, repetitive exposure to the CS in the absence of a US, gradually reduces the acquired fear response, a phenomenon called fear extinction. To identify the involved neuronal ensembles, we accomplished immunohistochemistry against the immediate early gene c-Fos, a marker of neuronal activity. We analyzed changes in neuronal activation patterns in key brain areas of the fear circuitry between fasted and non-fasted animals, exposed to fear extinction or without conditioning.

Interestingly, mice fasted during extinction learning displayed faster fear extinction than non-fasted controls, suggesting a direct relation between feeding and fear circuits in the brain. Fasting during the extinction process increased neuronal activation in the basolateral nucleus of the amygdala, a key structure of the fear response. In addition, we also detected changes in the central nucleus of the amygdala, a pivotal brain area for fear expression and in the paraventricular nucleus of the thalamus, a relay structure of sensory inputs.

These experiments suggest several brain structures as possible interaction sites between feeding and fear circuits. We are now planning to manipulate neuronal ensembles in these brain areas to elucidate their role during feeding and fear-dependent challenges.

Pharmacological investigations of 6-desoxo-N-methylmorphinans as new potent μ-opioid receptor agonists: binding, signaling and antinociceptive activity

Poster # 88

Pharmacology and Neuroscience

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PhD programme
Pharmaceutical Sciences

Pain is a major health problem, and a multidimensional phenomenon. Worldwide, ca. 20-30% people suffer from chronic pain. Opioid analgesics are the cornerstone drugs for the treatment of moderate to severe pain. However, they also produce several adverse effects. Pharmacological actions of opioids are primarily mediated through activation of the μ-opioid receptor (MOR). One long-standing focus of drug discovery has been the search for opioids exhibiting a favorable dissociation between analgesia and the occurrence of unwanted side effects. Among opioids, morphinans are of major importance as the most effective analgesics. The clinically relevant analgesic oxymorphone represents a valuable scaffold for the design of MOR ligands with representative examples including the 14-O-methyl and 14-O-benzyl substituted derivatives, and the 5-methyl substituted analogue, 14-methoxymetopon. Position 6 is one of the most manipulated sites, and established to play a key role on opioid activity in vitro and in vivo. The consequence of the deletion of the 6-carbonyl group in targeted N-methylmorphinans on ligand-MOR interaction, signaling and antinociceptive activity is presented. Radioligand binding studies showed that the 6-desoxy derivatives display affinities in the subnanomolar range at the human MOR, and are MOR selective. The loss of the 6-carbonyl group was not favorable when comparing oxymorphone and 14-methoxymetopon to their 6-desoxy counterparts, while a significant increase in MOR affinity and selectivity was observed for 6-desoxo-14-O-benzyloxymorphone. The 6-desoxo derivatives were very potent agonists with full efficacy in MOR-induced G protein coupling, with 6-desoxo-14-O-methyloxymorphone, 6-desoxo-14-O-benzyloxymorphone and 6-desoxo-14-methoxymetopon retaining or displaying an improved agonism than the parent compound, exception being 6-desoxooxymorphone. In vivo, the 6-desoxo derivatives were effective against acute thermal nociception in mice, with comparable or increased potency than the lead molecules. The absence of a 6-carbonyl group in targeted N-methylmorphinans has a strong influence on binding to the MOR and post-receptor signaling, with 6-desoxooxymorphone, 6-desoxo-14-O-methyloxymorphone, 6-desoxo-14-O-benzyloxymorphone and 6-desoxo-14-methoxymetopon evolving as potent MOR agonists in vitro and in vivo. These results expand the understanding of the impact of the 6-CO to 6-CH2 alteration on ligand-receptor interaction and molecular mode of action, and may aid in identification of new opioid analgesics with improved pharmacological profiles.

Poster # 89

Pharmacology and Neuroscience

Kappa opioid receptor activation, signaling and bias agonism exploration of differently substituted diphenethylamines as new kappa opioid antinociceptives

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The κ-opioid receptor (KOR) has a central role in modulating neurotransmission in neuronal circuits that subserve pain. KOR activation produces analgesia without causing dependence, euphoria or respiratory suppression. However, dysphoria, anhedonia and psychotomimesis are associated to KOR stimulation, thus precluding clinical development of therapeutics targeting the KOR. Physiological effects of KOR activation result from distinct signaling pathways: analgesia is G protein-mediated, and adverse effects are linked to the β-arrestin2 recruitment. The concept of biased agonism at the KOR has gained significant importance to drug discovery, with G protein-biased KOR agonists evolving as prospective analgesics with improved benefit/risk profile. Our research led to the discovery of a new molecular scaffold for KOR ligands within the class of diphenethylamines. Encouraged by the SAR outcomes on the functional activity of HS665 (agonist) and HS666 (partial agonist), a novel series of diphenethylamines as potential G protein-biased KOR ligands was designed. In vitro binding studies showed that the new diphenethylamines are KOR selective and display very high affinities at the human KOR, for several derivatives being in the picomolar range. They are highly potent in stimulating G protein coupling through the KOR, acting as full agonists or partial agonists. Several diphenethylamines showed significant ligand bias for G protein activation over β-arrestin2 recruitment at the KOR, based on the much lower potencies and efficacies in promoting β-arrestin2 signaling than in stimulating [35S]GTPγS binding. In vivo, they induced marked antinociception in a visceral pain model in mice, with comparable or increased potencies than the lead molecules. We established that affinity and selectivity at the KOR, efficacy and potency to activate KOR and bias agonism were strongly influenced by the nature of the substituent at the nitrogen or the position of the phenolic hydroxyl group. Analysis of the KOR functional activity of the new diphenethylamines provides valuable insights into their signaling pathways, with several ligands appearing to carry the pharmacological characteristics of G protein-biased KOR ligands. These results offer valuable structural and functional insights into the design and/or discovery of drugs targeting the KOR with improved pharmacological profiles and enhanced therapeutic efficacies for the treatment of pain.

Modulation of Fibroblast Growth Factor Receptor 1 signalling by Nogo-66 Receptor 1: relevance for peripheral axon regeneration

Poster # 90

Pharmacology and Neuroscience

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PhD programme
Signal Processing in Neurons (SPIN)

The Fibroblast Growth Factor (FGF) family comprises 23 members which can bind to four types of tyrosine kinase receptors (FGFR1-4). In the nervous system, FGF-2 and its high-affinity receptor FGFR1 are constitutively expressed by all motor and sensory neurons projecting into peripheral nerves in which FGF-2 up-regulation promotes their survival and enhances neurite outgrowth after a lesion. The effect of FGFR1 activation may involve its interaction with other receptors such as Nogo-66 receptor 1 (NgR1), a negative regulator of axonal growth. In fact, the ectopic over-expression of NgR1 blocks FGF-2-induced axonal branching in primary cortical neurons. Furthermore, a high-affinity binding of FGF-2 to NgR1 was demonstrated.
We aim to investigate by which mechanisms NgR1 modulates FGFR1 signalling and how this can be relevant for peripheral nerve regeneration. Although our in vitro experiments did not show an FGF-2-dependent effect on axon outgrowth in NgR1 knock-out DRG neurons as compared to wild type neurons plated on laminin (a growth promoting substrate), we are currently investigating the effect of the loss of NgR1, along with FGF-2 treatment, in an in vivo mouse model of sciatic nerve lesion. In fact, by taking advantage of double transgenic mice lacking NgR1 and expressing GFP in a small percentage of motor and sensory neurons, and by using light-sheet fluorescence microscopy, we will be able to evaluate the regeneration of single fibers in the transected sciatic nerve repaired with a collagen conduit. This model will allow us to assess axon regeneration in a more physiological environment and additionally to consider the effect on the motor component of peripheral nerves. It is known that FGF-2 selectively promotes motor neuron regeneration in spinal cord slices in vitro and in the sciatic nerve lesion model in vivo. Moreover, NgR1 contributes to macrophage clearance at the end of the period of Wallerian degeneration in injured peripheral nerves and we will analyse how this influences axon regeneration. In conclusion, this set of experiments will help in elucidating the role of NgR1 and FGFR1 signalling pathways in peripheral nerve regeneration.

Poster # 91

Pharmacology and Neuroscience

Role of the PACAP/PAC1 receptor system within the brain in the regulation of behavioral and neuroendocrine stress reactions

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PhD programme
Pharmaceutical Sciences

Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) is a neuropeptide that was first isolated from ovine hypothalamic extracts in 1989 with neurotransmitter, neurotrophic and neuroprotective properties. PACAP and its preferred receptor PAC1 have been shown to be expressed in brain areas involved in stress and anxiety responses such as the hypothalamic paraventricular nucleus (PVN), lateral septum (LS), amygdala and bed nucleus of stria terminalis (BNST). Intracerebral PACAP administration showed a direct functional interaction with corticotropin-releasing factor (CRF), the main activator of the neuroendocrine stress axis. Moreover, PACAP infusions into the cerebral ventricles lead to behavioral changes that can be observed after stress exposure. However, despite the evidence of an implication of the PACAP/PAC1 receptor system in stress mechanisms, there has been no direct functional evidence for an action of endogenous PACAP in distinct forebrain area on stress responses under ethologically relevant conditions. For instance, the specific role of PACAP/PAC1 receptor system on HPA axis regulation under stress conditions is still unknown. Therefore, aim of the present study was to investigate the role of the PACAP/PAC1 receptor system on neuroendocrine and behavioral stress reactions. We administered a PACAP agonist (PACAP-38) or an antagonist (PACAP 6-38) bilaterally into the PVN, LS or BNST of male Sprague-Dawley rats and tested animals in stress and anxiety-related behavioral tasks such as the forced swim or elevated plus-maze test. In addition, we measured ACTH and corticosterone levels before, during and after stress exposure. So far, we found that intra-PVN and intraseptal administration of the PACAP agonist significantly increased the immobility time and reduced active coping behavior during the forced swim exposure as PACAP-38 treated animals showed enhanced floating and reduced struggling behaviour compared to controls. Thus, our data showed that the PACAP/PAC1 receptor system in the PVN and LS is critically involved in the regulation of behavioral stress function.

Differentiation of human induced pluripotent and induced neural stem cells towards oligodendroglial fate

Poster # 92

Pharmacology and Neuroscience

Vanishing white matter disease (VWMD) is an autosomal-recessive hypomyelinating disease. As of today patients can only be treated symptomatically and die within a few years after disease onset. Therefore, cell based therapy involving patient-derived glial precursor cells offers a new approach in VWMD treatment. In order to achieve further progress in cell-based therapy, we aim to differentiate human induced pluripotent stem cells (hiPSCs) as well as induced neural stem cells (iNSCs) towards glial progenitor cells to generate transplantable glial precursor cells (GPCs).
In this study, diverse NSC and iNSC lines, obtained either through neural induction of hiPSCs or through direct conversion of fibroblasts, were assessed regarding their differentiation potential towards oligodendrocyte precursor cells (OPCs). So far it is still unknown whether in vitro generated iNSCs are capable to form OPCs and more mature oligodendrocytes. To achieve this, we followed two protocols and started differentiation from the NSC stage. Along the differentiation steps, cells of the oligodendrocyte lineage can be identified by gradually acquired markers. After a 5-day adaption phase, expression of NSC markers such as Nestin, Pax6, Sox1, Sox2, CD133, KI67 as well as OPC markers such as Olig2, Nkx2.2, PDGFRα were assessed through IF and qPCR. After at least 10 days maturation, a PDGFRα-positive-enriched progenitor population was observed with ongoing maturation as judged by O4 staining and morphology. Moreover, we applied a differentiation protocol that aims to generate an adherent and self-renewing population of radial glia like neural precursor cells (RGL-NPCs) as stable intermediate population, primarily giving rise to oligodendrocytes. First, we aimed to adapt NSC and iNSC lines to the RGL-NPC state. For differentiation, RGL-NPCs were cultured for 15 days in order to obtain Olig2, PDGFRα, NG2-positive progenitor cells. After a second differentiation step, the resulting OPC population was analyzed for NSC, RGL markers, as well as for pre/immature oligodendroglial markers such as O4, Nkx2.2, and Olig2 with immunocytochemistry and qPCR.
Presented results indicate for the first time oligodendroglial differentiation potential of induced neural stem cells, making them a suitable candidate for cell-based therapy with patient-derived glial precursors in the future.

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Poster # 93

Pharmacology and Neuroscience

Receptor tyrosine kinase trafficking in response to Sprouty2 regulation

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PhD programme
Neuroscience

Receptor tyrosine kinases (RTKs) control a variety of processes in the nervous system. The Sprouty (Spry) proteins belong to a group of evolutionary conserved modulators of RTK signaling. Spry2 inhibits extracellular signal-regulated kinase (ERK) signaling in response to fibroblast growth factor (FGF) or nerve growth factor (NGF). In contrast, Spry2 promotes ERK signaling induced by epidermal growth factor (EGF) and inhibits the transfer of EGF receptor (EGFR) from early to late endosomes.
The purpose of this study is to determine how the modulation of Spry2 levels influences internalization, trafficking and degradation of FGF receptor 1 (FGFR1) in comparison to EGFR in U373 glioma cells and to tropomyosin receptor kinase A (TrkA) in adult sensory neurons. Thus, we will analyze the effects of Spry2 on trafficking of different RTKs in the nervous system.
U373 glioma cells were transfected with ON-TARGET plus siRNA against Spry2 and its downregulation was determined by Western Blotting. Colocalization of FGFR1 with early endosome antigen 1 (EEA1), transferrin and lysotracker was analyzed in U373 glioma cells in response to downregulation of Spry2. Images were acquired by confocal microscopy and colocalization was analyzed using Imaris software.
RESULTS: Western Blot analysis confirmed downregulation of Spry2 protein levels by Spry2-siRNA. Colocalization analysis revealed that downregulation of Spry2 promotes colocalization of FGFR1 with early and recycling endosomes, whereas it reduces colocalization of FGFR1 with lysosomes.
Our preliminary data from U373 glioma cells reveal for the first time an effect of Spry2 on trafficking of FGFR1, which seems to be different from its role in EGFR transport. Therefore, we will carefully compare trafficking of FGFR1 and EGFR in glioma cell lines with stable overexpression or downregulation of Spry2. Furthermore, we will investigate the effects of Spry2 on trafficking of FGFR1 and TrkA in cultures of adult sensory neurons from heterozygous and homozygous Spry2 knockout mice. The results of this study will provide new insights into the effects of Spry2 on trafficking of different RTKs in the nervous system.

Towards an iPSCs based model of multiple system atrophy

Poster # 94

Pharmacology and Neuroscience

The goal of the project is to develop a complimentary humanized in vitro model by differentiating induced Pluripotent Stem Cells (iPSCs) from MSA patients to oligodendrocytes.
Multiple System Atrophy (MSA) is a fatal progressive neurodegenerative disease featuring motor and autonomic symptoms. The neuropathological hallmark of MSA is the abnormal accumulation of alpha-synuclein in oligodendrocytes. The diseases mechanisms are poorly understood and animal models replicate only mechanistically pathogenic cascades relevant to MSA.
iPSCs were subjected to differentiation towards oligodedendrocytic fate with a modified protocol of Douvaras et al 2013.
After 72 days of iPSCs controlled differentiation CNPase positive cells were observed.
To this end we were able to achieve the stage of late oligodendrocyte precursor cells after human iPSCs differentiation.

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PhD programme
Neuroscience

Identification of the immunodominat T cell epitopes of AQP4 and MOG in demyelinating diseases

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T-cells, especially CD4+ T-cells, are key players in the pathogenesis of autoimmune diseases, which mediate cellular and humoral immune responses. Autoantibodies targeting the aquaporin-4 (AQP4)-water-channel-protein and the myelin-oligodendrocyte-glycoprotein (MOG) are associated with a broad spectrum of human CNS demyelinating diseases including neuromyelitis optica spectrum disorders (NMOSD) and acute disseminated encephalomyelitis (ADEM). Whereas there is some information on the role of AQP4-specific T-cells, little is known about MOG-specific T-cells in these diseases. We therefore aimed to identify the immunodominant T-cell epitopes of AQP4 and MOG in patients with NMOSD.

We performed a T-cell epitope mapping of human AQP4 and MOG peptides using the CFSE-proliferation assay. Peripheral blood mononuclear cells (PBMCs) of eight AQP4-antibody and four MOG-antibody positive NMOSD patients, one MOG-antibody positive ADEM patient and 10 healthy controls were stimulated with a library of eight AQP4 and nine MOG peptides. After eleven days, the proliferation of PBMCs in response to single peptides via the dilution of the CFSE-staining was analysed by flow cytometry. Furthermore, the cytokine secretion, particularly granulocyte macrophage colony-stimulating factor (GM-CSF) and interferon (IFN)-γ was examined using ELISA. For investigating the differentiation of T-cells into distinct CD4+ T helper cell subsets, particularly Th1 and Th17 cells producing proinflammatory IFN-γ and interleukin (IL)-17, respectively, a fluorescence-cytometry-based intracellular staining was performed.

We detected higher peptide specific T-cell proliferation in response to AQP4 peptides in all NMOSD patients when compared to healthy controls. A T-cell response to MOG peptides, preferably to peptides corresponding to the extracellular immunodominant Ig-domain, was found in NMOSD patients as well as in healthy controls. The production of cytokines (GM-CSF and IFN-γ) correlated with T-cell proliferation.

To conclude, our study indicates a specific T-cell response to AQP4, but not to MOG, in patients with NMOSD. We expect that our results are important for the development of new individualised immune tolerance therapies.

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PhD programme
Neuroscience

A $Ca_V1.3$ L-type Ca^{2+}-channel splice variant stabilizes more negative activation voltages

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$Ca_V1.3$ voltage-gated L-type Ca^{2+}- channels (LTCCs) activate at more negative voltages than other high-voltage gated Ca^{2+} channels which, in contrast to other LTCCs, allows them to support pacemaking activity in the sinoatrial node, hearing function in cochlear inner hair cells (IHCs) and neuronal firing. Negative activation is critically controlled by alternative splicing in the $Ca_V1.3$ C-terminus, but the contribution of alternative splicing for channel gating in other channel domains are largely unexplored. Evidence from a stable $Ca_V1.3$ cell line indicate a possible role of a combination of exons 8b, 11 and 32 for more hyperpolarized activation voltages.

We therefore transfected tsA-201 cells stably expressing auxiliary β_3 and $\alpha_2\delta$-1 subunits with $Ca_V1.3$ α_1-subunit ($Ca_V1.3_S$, short C-terminus) splice variants containing (mutually exclusive) exon 8a or 8b with or without exons 11 and 32 and performed whole-cell patch-clamp recordings with 15 mM Ca^{2+} as a charge carrier. Expression of these exons in various tissues was tested by PCR.

The voltage-dependence of activation and inactivation was shifted by ~3 mV towards more hyperpolarized voltages in $Ca_V1.3_S$ containing additional exons 11 and 32 compared to $Ca_V1.3_S$ lacking these exons. Furthermore, the combination of exons 8b, 11 and 32 showed a more pronounced shift of steady-state inactivation to more negative potentials compared to the combination containing exons 8a, 11 and 32. PCR experiments revealed expression of exons 8a, 8b, 11 and 32 in mouse whole brain and identified transcripts containing exon 11 and 32 together.

We propose that in addition to C-terminal splicing also alternative splicing in exons 11 and 32 contributes to the stabilization of negative activation voltages of $Ca_V1.3$ channels. This mechanism may be important to regulate $Ca_V1.3$ activity in neurons.

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PhD programme
Molecular Cell Biology and Oncology (MCBO)

Circuitry of NPY-expressing Neurons in the Dorsal Hippocampus

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PhD programme
Neuroscience

Neuropeptide Y (NPY) is highly enriched in limbic brain areas and well known for its anxiolytic and fear-suppressing properties. These effects are mediated predominantly by Y1 receptors. The role of the extensively expressed pre-synaptic Y2 receptors (Y2R) is, however, not clear yet. In addition to investigating the role of NPY acting on Y2R in emotional and non-emotional learning, we were interested in the expression and projection patterns of NPY neurons in the hippocampal dentate gyrus.
We combined cre-dependent viral vectors with transgenic mouse lines and immunohistochemical methods to identify different populations and synaptic targets of NPY-expressing neurons of the hippocampus.
For anterograde tracing, mice expressing cre-recombinase under either the Parvalbumin (Pvalb) or Somatostatin (Som) promoter were injected unilaterally into the dorsal dentate gyrus with a flexed viral vector expressing the fusion protein SypGFP to identify projections of these GABAergic neurons.
For retrograde tracing of NPY neurons, the fluorescent tracer FluoSpheres® was injected either into the dorsal hippocampus or the medial septum of NPY-GFP transgenic mice, to identify whether NPY expressing neurons project from the dentate gyrus to the medial septum or vice versa.
Immunohistochemical analysis revealed co-expression of NPY with Pvalb as well as Som, but not Calretinin in the mouse dentate gyrus. Furthermore, in addition to their local function as interneurons, both Som- as well as Pvalb-neurons of the dentate gyrus were sending projections to other brain areas, such as the medial septum.
Our findings confirm largely what is known from literature, however the co-expression of NPY and Parvalbumin in mouse hippocampus GABAergic neurons has not yet been described.
One pitfall however may be the Pvalb-cre mouse, as vector expression can be seen in brain areas associated with mossy cell projections, thus raising the question whether Pvalb may be expressed at sub-detection levels also in glutamatergic neurons. To address this, we will produce a viral vector that is not only cre-dependent but has a promoter active only in GABA neurons to confirm whether the projections we observed are indeed from Pvalb-positive GABAergic neurons.

The effect of Spry2 and PTEN downregulation on axon growth

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PhD programme
Neuroscience

Axon growth during development and regeneration is influenced by growth factors. By activation of receptor tyrosine kinases (RTKs), growth factors stimulate different signaling pathways including the Ras/extracellular signal-regulated kinase (ERK) and the phosphatidylinositol-3-kinase (PI3K)/Akt pathway. Sprouty2 (Spry2) has been known as a negative regulator of the Ras/ERK pathway whereas phosphatase and tensin homolog deleted on chromosome 10 (PTEN) acts mainly as an inhibitor of the PI3K/Akt pathway. Adult sensory neurons with downregulation of Spry2 by shRNA as well as sensory neurons from Spry2 deficient mice showed significant increases in axon growth. Knockdown of PTEN increased axon growth of sensory neurons as well, and this effect was further enhanced in pre-lesioned neurons. Therefore, the aim of the present study is to analyze axon growth of adult sensory neurons in response to downregulation of both, Spry2 and PTEN, to elucidate possible additive effects of these signaling regulators on axon outgrowth.
Dorsal root ganglia (DRG) from wild-type, heterozygous Spry2 (Spry2+/-) and homozygous Spry2 (Spry2-/-) deficient mice were harvested and used for RNA extraction or cell culture experiments. The expression of PTEN mRNA was analyzed using qPCR. For PTEN mRNA downregulation, adult DRG cultures were transfected with Accell PTEN siRNA. Axon growth and PTEN protein levels were analyzed after growth factor treatment using immunostaining for Tuj1 and PTEN.
PTEN mRNA levels were not changed in DRG tissue of Spry2+/- and Spry2-/- compared to the wild-type. Accell PTEN-siRNA induced 79% downregulation of PTEN mRNA after 72h in adult DRG cultures. Reduced PTEN protein levels of DRG neurons in response to PTEN-siRNA were detected by immunostaining after 72 h. The preliminary results revealed increased axon growth in response to PTEN downregulation with or without growth factors and this effect seems to be more prominent in Spry2+/- neurons than in wild-type neurons.
In summary, our preliminary data indicate that downregulation of both, Spry2 and PTEN, promotes axon growth of adult sensory neurons in an additive manner.

Role of BDNF and neurotrophic receptors in human inner ear development

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The expression patterns of the neurotrophin, Brain derived neurotrophic factor, BDNF as well as the neurotrophic receptors - p75NTR and Trk receptors in the developing human fetal inner ear between the gestational weeks (GW) 8 to 12 is examined via in situ hybridization and immunohistochemistry.
BDNF mRNA expression elevates at the earliest stage examined within the Kölliker's Organ (KO) and the Greater Epithelial Ridge (GER) following which the expression declines. p75NTR immunostaining is most prominent amongst the nerve fibers which further penetrate into the KO and the macular organs as gestational age progresses. TrkB & TrkC expression intensifies towards GW12 at which point the BDNF mRNA localization is at its lowest point. TrkA expression is limited to regions of the facial nerve at GW10. Unlike previous results from embryonic murine specimens we could not observe a significant BDNF gradient along the tonotopic axis although the expression declines as gestational age progresses. This contrasts with the apparent increase in TrkB expression at GW12 indicating a significant decline in neurotrophic support as gestational age progresses.

Retinal morphology in a mouse model of multiple system atrophy

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Neurodegenerative diseases like Parkinson's disease (PD) and Multiple System Atrophy (MSA) have been shown to exhibit physiological and morphological neuronal abnormalities. These abnormalities can result from exceeding aggregation of α-synuclein (α-SYN), a 140aa presynaptic protein that is involved in vesicular transport. Both PD and MSA are associated with a variety of visual symptoms. We therefore aimed at investigating the underlying mechanisms of α-SYN aggregation in homozygous transgenic mice overexpressing human α-SYN under the proteolipid protein (PLP)-promoter (PLP-α-SYN) compared to wild type (WT) animals of two different age groups (two months, one year). By performing immunohistochemical analyses on vertical retinal sections we discovered distinct α-SYN signal occurring in different retinal cell layers of PLP-α-SYN mice, but not in WT mice. This is remarkable because the PLP promotor driving the α-SYN expression in oligodendrocytes was reported to be inactive in the retina. Our PLP-stainings confirmed that the expression stops at the optic nerve/retina junction, where we observed a colocalization with α-SYN. To confirm the expression of PLP driven expression of α-SYN, we performed quantitative real-time PCR using specific TaqMan® probes to detect expression levels of PLP1, α-SYN and specific PLP-α-SYN construct mRNA in retinal tissue, using whole brain extract as positive control and liver extract as negative control. More immunohistochemical experiments included the investigation of the glial fibrillary acidic protein (GFAP), a marker for activation of Müller glia that can indicate neuroinflammatory processes that could occur as a result of neurodegeneration; Iba1, a marker for microglial activation and tyrosin hydroxylase that labels dopaminergic neurons. GFAP-positive fibers spanning the peripheral retina were pronounced in aged animals in WT and even more in PLP-α-SYN suggesting enhanced neuroinflammation in the MSA animals. In PLP-α-SYN animals, tyrosin-hydroxylase-positive processes appeared to reach deeper strata of the inner plexiform layer, and cell bodies were deformed. Analyses of TH stainings using a MatLab script depicted more TH signal in MSA animals, which will be further investigated using qRT-PCR. Further investigations will be needed to clarify whether defects in retinal morphology could be exploited as differential diagnostic marker in MSA.

Poster # 101

Pharmacology and Neuroscience

Extracellular levels of catecholamines are altered in the medial prefrontal cortex of non-extinguishing S1 mice

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Despite its success in treating specific anxiety disorders, the efficacy of exposure-based therapy is limited in terms of duration and the occurrence of fear relapse after initial response. Enhancing dopaminergic signalling with the dopamine bioprecursor L-DOPA promotes successful long-term fear extinction, the central mechanism of exposure-based therapy, in healthy subjects as well as it initiates short-term extinction memory of cued conditioned fear in the extinction-deficient 129S1/SvImJ (S1) mouse. In an attempt to identify the brain regions, that mediate the extinction-enhancing effect of L-DOPA, we performed a microinjection study targeting the medial prefrontal cortex (mPFC); a key brain region for the retrieval of the extinction memory. Local application of dopamine into the mPFC prior to extinction training caused the formation of a persistent and context-dependent fear extinction memory raising the idea that a dysfunctional dopaminergic neurotransmission may underlie the extinction deficits observed in S1 mice. Therefore, we next investigated the dynamic changes in extracellular levels of dopamine using in-vivo microdialysis and high-performance-liquid-chromatography (HPLC). First results show that cued fear conditioning caused a pronounced and prolonged dopamine release in the mPFC of extinction-competent BL6 mice. In contrast, the local increase in dopamine release following conditioning was transient in S1 mice. On the next day, dopamine levels were reduced in S1 mice during all phases (prior to, during and after) of extinction training as compared with control Bl6 mice pointing towards blunted dopamine neurotransmission in S1 mice. Taken together, the present findings suggest that increasing dopamine availability may be a promising strategy to boost the extinction of learned fear in a clinically relevant model that mimics a group of patients suffering from an anxiety- or trauma-related disorder.

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PhD programme
Signal Processing in Neurons (SPIN)

Rewiring of cone bipolar cells in a mouse model of congenital stationary night blindness type 2

Poster # 102

Pharmacology and Neuroscience

In humans, mutations in the CACNA1F gene which encodes Cav1.4 L-type calcium channels are associated with congenital stationary night blindness type 2 (CSNB2). Cav1.4 channels which are predominantly expressed at photoreceptor (PR) terminals and most likely also in bipolar cells (BC). They allow sustained calcium influx and ensure neurotransmitter release. Moreover they serve an important role for PR synapse formation and maturation. The retinal morphology in Cav1.4 mouse models (Cav1.4-KO, loss-of-function; Cav1.4-IT, gain-of-function) showed structural remodelling of rod BCs. Dendrites of rod BCs elongated into outer nuclear layer (ONL), the organisation of the outer plexiform layer was disrupted and the structures of PR synaptic ribbons had variable, mostly immature appearance. However it was unclear whether rod BCs formed new connection with displaced PR terminals or these reflect mislocated existing contacts because of rod spherule migration. Labelling with PSD95 showed that in Cav1.4-IT retinas some synaptic terminals were mislocated in the ONL (n=5; 5-6 and 13-14 week-old mice). Mislocalized terminals contained mostly immature ribbons as seen in co-staining with the ribbon marker CtBP. Only some rod BC dendrites approached those terminals but never formed an invaginating contact suggesting that rod BCs try to form new connections. Cav1.4-KO mice ribbons showed only circular appearance but PSD95 protein was undetectable (n=5; 5 and 14 week-old mice). This finding confirmed the crucial role of Cav1.4 channels for the formation of synaptic structures and suggested that different CSNB2 mutations cause different types of morphological aberrations. Preliminary immunohistochemical analyses of Cav1.4-IT retinas using secretagogin (SCGN) as a marker for Type 2-6 and possibly Type 8 cone BCs elicited that some cone BCs showed elongated dendrites in the ONL. This phenotype was seen at the age of 5 and 14weeks (n=5). To determine whether all SCGN stained BCs or just some are affected, we will investigate additional specific markers such as PKARIIβ to identify Type 3b cone BC, calsenilin for Type 4 and CaBP5 Type 3 and 5. Taken together our immunohistochemical analysis will elicit further mechanisms by which Cav1.4 channel dysfunction affects retinal morphology.

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PhD programme
Pharmaceutical Sciences

Functional neuroanatomy of prodynorphin

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PhD programme
Signal Processing in Neurons (SPIN)

Dynorphins (Dyn) and kappa opioid receptors (KOR) are abundantly expressed throughout limbic brain areas and were shown to be involved in the regulation of anxiety and stress control. Moreover, the Dyn/KOR system is implicated in the pathophysiology of depression, anxiety and addiction. However, the organization of the Dyn/KOR system is highly complex

Understanding and potentially interfering with this complex system to target specific functions depends on a detailed understanding of specific functional roles of individual dynorphinergic neurons as well as neuronal population. For this goal, we implemented independent, yet complementary startegies based on restricted prodynorphin (pDyn) knock-out (achieved so far in the bed nucleus of the stria terminalis (BNST), the central nucleus of the amygdala (CeA) or NKB-expressing cells) or re-expression (in the BNST). Testing these mice in paradigms related to anxiety (open-field, elevated plus maze, light-dark choice test) and stress-coping behaviour (tail suspension test) did not reveal significant differences between the investigated groups.

By contrast behaviour in the fear conditioning and extinction paradigm was altered. Deletion of pDyn in the BNST resulted in the delayed fear extinction. No differences were observed upon deletion of pDyn in the CeA.

We also employed the cocaine-induced conditioned place preference paradigm to investigate the extinction and stress induced reinstatement of the place-conditioned response. Mice with deletion of pDyn in NKB-positive cells displayed no stress-induced reinstatement, while control mice did.

Our data so far revealed first indications for regional differences in Dyn functions. These are in line with the known role of Dyn in fear and anxiety and stress control. Further studies on the role of Dyn in specific brain areas in addictive process and fear extinction are in progress.

Impact of Autonomic Movement on Adverse Events in Relaxing Virtual Environments

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PhD programme
Psychology

Virtual Reality (VR) is increasingly used in clinical psychology to support established therapeutic techniques. Mainly used for the treatment of post-traumatic stress disorder (PTSD) and specific phobias, current studies imply that VR may also be a feasible tool to induce relaxation. However, VR comprises several potential challenges (e.g. discomfort or motion sickness), which may not only be related to the content of the virtual environment (VE), but also to the degree of autonomic movement in the VE. The aim of the study was to assess the frequency of adverse events in a relaxing VE and factors associated with these.

The study comprised three short virtual scenarios: (a) no movement of the avatar, (b) steady non-autonomic movement, and (c) autonomic movement. Adverse events (e.g. nausea, headache, dizziness) were assessed using a 4-point rating scale. As potential factors being associated with the occurrence of adverse events we assessed general vulnerability of nausea or dizziness in everyday life, and attitudes towards and use of modern technologies.

We included a sample of 30 healthy participants (mean age: 39.5 years, range 23-63 years; 60% female) in the study. Most frequent adverse events were dizziness (77.7%), nausea (46.7%) and discomfort (43.3%). While the majority of participants reported no or very little adverse events in the first two scenarios, most participants reported at least mild adverse events in scenario C. General vulnerability of nausea or dizziness in everyday life was correlated to the intensity of adverse events ($r= 0.63$, $p<0.001$), but no correlations were found with the participants' age, use of or attitudes towards modern technology.

Virtual Reality represents an innovative and promising extension of traditional psychological techniques. The results of the present study indicate that autonomous movement in the VE may result in motion sickness in a substantial number of participants. To prevent the occurrence of such adverse events we suggest to use non-autonomic movement.

Poster # 105

Pharmacology and Neuroscience

Voltage-gated sodium channel distribution in human cochlea and other mammalian species

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PhD programme
Neuroscience

The restoration of the hearing system by cochlear implants has its limitations compared to acoustic hearing. To overcome these restrictions it will be necessary to unravel the ion channel composition in the human inner ear. Voltage-gated sodium channels (Nav) are essential for the initiation of the action potential (AP) in excitable cells and likely are responsible for AP generation through electrical stimulation (ES).
We have mapped Nav channel composition in man and other selected mammalian cochleae. The hearing organ transforms sound into an electrical signal by subtypes of local neurons that enable the enormous dynamical range of the primary receptor cells. A characterization of these neurons shall provide normative data and basis to model AP initiation by ES.
Immunohistochemical techniques along with western blot analysis of inner ear tissue were used to detect Nav channels in human, mice, rats, guinea pigs and cats. Nav 1.6 was the most widespread voltage-gated sodium channel in the afferent innervation in human and mice. It was located in spiral ganglion neurons (SGNs), its heminodes, nodes of Ranvier and intermediate cells of the stria vascularis. There were other Nav channels such as Nav 1.7 which was highly expressed in the inner hair cells and SGNs. Moreover, it was present in Ranvier nodes in human. Nav 1.1 was present in non-myelinated nerve fibers in the osseous spiral lamina and in the outer and tunnel spiral bundle in human cochlea. Due to this pattern of expression, Nav 1.1 may be part of the efferent innervation including the medial efferent system and maybe the lateral efferents. Nav 1.3 was present in mice and human SGNs.
The differential expression of these channels and the differences in their biophysical characteristics may define several neuronal subpopulations with specific functions and properties in the hearing process. If we better understand the distribution of different subpopulations of neurons starting with a mapping of the Nav channel heterogeneity we may be able to fine tune ES with an implant in man. The generation of these normative data for the human hearing organ is relevant to further analyze pathological changes and deterioration with age.

Audio and 3D-Visual guidance for optimal placement of an Auditory Brainstem Implant with magnetic navigation and maximum clinical application accuracy

Poster # 106

Pharmacology and Neuroscience

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PhD programme
Image-guided Diagnosis and Therapy

This work presents a dynamic 4D (audio and 3D-visual) feedback system for positional guidance in real-time during surgical placement of an Auditory Brainstem Implant.
At present, dummy and simultaneous measurements, usually including the MRI and/or CT scans of the patient´s head, are used to determine the optimal position for an Auditory Brainstem Implant (ABI) on the nucleus cochlear. When found, the optimal position is marked by the surgeon and in the next phase the surgeon tries to locate the optimal position in the patient´s head and place the implant.
With current technology there are no quantifiable methods for storing the optimal implant position. Among other things, brainstem implants do not always provide satisfying and predictable results in hearing perception. Therefore, the aim is to equip the surgeon with a new navigation system based on magnetic navigation and maximum clinical application accuracy; to provide intuitive audio and 3D-visual guidance for positioning the implant; to store the optimal position of the implant in a database and to reevaluate its optimal position.
The navigation software will be platform-independent and developed in the C++ programming language. The basis for this software is Rhinospider Technology developed by the University Clinic for HNO at the Medical University of Innsbruck. One of the goals is to get the certification of this software as a medical device. This project would be paving the path for intraoperative support during ABI procedures.

Impaired fear extinction is associated with a dsyregulation of microRNAs in the amygdala

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Post-Doc

MicroRNA (miRNA)-mediated control over the expression of learning and memory-related genes suggest miRNAs as interesting candidates for novel targets and/or as biomarkers in the treatment of anxiety disorders, where specific learning mechanisms are adversely affected. Using 129/SvlmJ (S1) mice, an inbred mouse strain displaying impaired fear extinction memory formation, we aimed to gain insight into miRNAs and the role they play in impaired fear extinction.
To gain insight into a possible dsyregulation of miRNAs in mice with fear extinction deficits, we performed small RNA sequencing between impaired S1 and normally extinguishing 129S6 (S6) mice. We revealed 197 dsyregulated miRNAs in the amygdala of S1 mice following fear extinction training. There were 96 miRNAs that displayed lower expression and 101 miRNAs that exhibited higher expression. Among these regulated miRNA candidates, were the higher expression of the fear-associated miR-182. Confirming the higher expression of miR-182 in impaired S1 mice and examining the regulation of miR-182 during the expression of fear we reveal that, only following successful fear extinction, the expression of miR-182 is decreased suggesting a learning induced decrease in the expression of miR-182. We next explored the spatial localization of miR-182 in the amygdala during fear extinction training and assessed the implications of miR-182 downstream targets in successful fear extinction. Taken together, these results highlight fundamental differences in miRNOME expression between extinction-impaired S1 and extinction-intact S6 mice suggest miR-182 as a potential target for the treatment of anxiety- and fear-related disorders such as PTSD.

Proangiogenic neuropeptides in experimental choroidal neovascularisation

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PhD programme
Molecular Cell Biology and Oncology (MCBO)

Wet age-related macular degeneration (AMD) is characterised by the formation of choroidal neovascularisations (CNVs) causing macula edema and/or bleeding resulting in central vision loss. Vascular endothelial growth factor (VEGF) is known to be the major involved growth factor in choroidal angiogenesis. In this study we aim to evaluate 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.
Volumes of laser-induced CNV spots from substance P receptor (nk1r) and npy receptor (y2r) knockout (k.o.) mice were compared to C57bl6/N wildtype (wt) mice. Further wildtype mice were injected their neuropeptide receptor antagonist intravitreally directly after laser treatment as well as at the peak of CNV development and volumes were compared to sham injections. CNV spots were analysed with optical coherence tomography (OCT) to obtain overall CNV volumes and flatmount preps for vessel volumes only.
In OCT CNV spots from nk1r k.o. mice (n=35) showed smaller overall volumes compared to spots from wt (n=50) mice (p=0.031). In flatmount preps the vessel volumes of both nk1r- (n=35) and y2r k.o (n=44) mice showed smaller volumes compared to wt mice (n=50, p<0.001, p<0.001, respectively). On the development of CNVs the injection of the y2r antagonist showed smaller overall and vessel volumes (n=14, n=19) compared to sham injected mice (n=12, n=16, p=0.013, p=0.021, respectively). The neuropeptide receptor antagonists showed no difference on the regression of CNVs volumes compared to sham injections.
The smaller CNV volumes in the knockout mice prove the involvement of the investigated neuropeptides in experimental choroidal neovascularisation. The y2r antagonist was able to reduce the volume of laser-induced neovascularisations in the development of CNVs, but showed no effect on the regression of fully developed CNVs, indicating an early contribution of NPY in experimental neovascularisation.
It is currently being investigated if there is an additive antiangiogenic effect when combining the neuropeptide receptor antagonists with the state-of-the-art anti-VEGF therapy on the development and regression of laser-induced CNVs.

Sprouty2 controls VEGF expression and tumorigenic capacity of glioma cells

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PhD programme
Signal Processing in Neurons (SPIN)

Glioblastoma multiforme (GBM) is the most malignant brain tumor. Accumulating evidence shows that GBM harbors a subset of cancer stem-like cells (CSCs) with tumorigenic capability. In this regard, a better understanding of the underlying molecular mechanisms that confer the phenotypic features of stem-like GBM cells may be crucial to target CSCs.
As a negative regulator of receptor tyrosine kinase (RTK) signaling, sprouty (SPRY) protein was first identified in Drosophila. On the contrary, mammalian SPRY2 also enhances EGFR signaling by inhibiting Cbl-mediated endocytosis of EGFR. SPRY2 deregulation has been shown to be involved in most cancers and its effect on malignancy depends strongly on cancer-type. The function of SPRY2 in GBM, however, is incompletely understood. Here, we report that SPRY2 is up-regulated in malignant glioma and correlates with reduced survival in glioma patients.
We investigated how SPRY2 affects stem-like GBM cells in spherogenic culture conditions. As a result, SPRY2 knockdown impaired significantly tumorsphere formation of glioma cells, and STAT3 was necessary for SPRY2 to promote tumorsphere formation in glioma. In addition, our data demonstrate that SPRY2 was required for tumor propagation in xenografts in vivo. We further found that SPRY2 induced VEGF expression in glioma cells. In conclusion, the present study highlights a tumorigenic potential of SPRY2 that is based on the regulation of VEGF, suggesting that SPRY2 may be a promising therapeutic target for GBM.

Role of Glucocorticoids on dendritic spine plasticity during onset and progression of Alzheimer's Disease in 3xTg-AD mice

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PhD programme
Neuroscience, Psychological and Psychiatric Sciences

The Glucocorticoids hormones act on brains, through mineralcorticoid and glucocorticoid receptors (GR), regulating physiological and behavior responses under baseline conditions and after stress. Glucocorticoids hormones are involved in Alzheimer's Disease (AD) and other neurodegenerative diseases. The aim of our research is to assess the role of glucocorticoids in the pathogenesis of AD and, in particular, to evaluate how the administration of agonists and antagonist of glucocorticoid receptors (GR) alter synaptic plasticity, causing a remodeling of dendritic spines in an AD mouse model (3xTg).
We administered either dexamethasone, an agonist of GR, or mifepristone, an antagonist of GR, to 6 and 10 month old 3xTg male mice. After perfusion with paraformaldehyde, brains were processed for Golgi Cox staining to highlight neurons and dendritic spines. We acquired images in bright field, using Neurolucida software, reconstructed the dendrites and calculated the dendrite spine density using the software Imaris (Bitplane).
Our results show that treatment with GR antagonist lead to a 23% reduction of spine density in CA1 region of hippocampus during the onset of AD, whereas mifepristone resulted in a 14% and 10.5% significant increase respectively in 6 and 10 months old. Dexamethasone treatment, at 10 months old, suggest comparable results with early phase, but now we are completing the analysis.
Our results shown that dexamethasone-dependent reduction of spines in an AD animal model is bigger than the one found in literature for treated wild type mice. Moreover, we know from literature that administration of dexamethasone in 3xTg mice leads to a major production of Abeta and Tau. Together, these data suggest a synergy between the molecular pathways leading to AD and the molecular action of glucocorticoids. Considering our results obtained with mifepristone treatment, the blocking of GR could represent a promising therapeutic approach to slow down the onset and the progression of AD.
In further studies, we will investigate the molecular mechanism underlying the dendritic spine density changes using in vitro techniques.

Instrumental learning retrieval of appetitive memory reconsolidation in rats

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PhD programme
Neuroscience, Psychological and Psychiatric Sciences

Reconsolidation, the process that restabilizes a previously consolidated memory after its reactivation, is considered a window of lability during which pharmacological or behavioural manipulations can interfere with the restabilization of the memory, enhancing or weakening its strength. While the occurrence of Pavlovian memories reconsolidation has been widely demonstrated, it is still debated whether instrumental memory can undergo reconsolidation after its reactivation.
The aim of our project was to provide an experimental evidence of food instrumental memory reconsolidation through a molecular assessment.
We initially trained rats to instrumental food self-administration for 10 days. Then, rats remained in forced abstinence for 14 days and, 24 hours later, one group was re-exposed to a non-reinforced retrieval session and the other to a no-retrieval session, in the training context. After 2 hours we analyzed the expression of GluN2B-containing NMDA receptors (GluN2B-NMDARs) and zinc finger protein 268 (Zif268; a validated markers of reactivation), and of phosphorylated ribosomal protein S6 (rpS6P; a relatively novel marker of memory reconsolidation), in rat key brain areas.
Behavioural study did not show significant differences on food-seeking behaviour for rats exposed to retrieval compared to no-retrieval. However, molecular analysis showed a significant increase of total GluN2B-NMDARs in amygdala after retrieval, supporting the trigger of memory destabilization after reactivation. As further confirmation, Zif268 protein expression increased after retrieval in the nucleus accumbens shell (NAc Shell), central (CeA) and basolateral amygdala (BLA). rpS6P increased in NAc Shell and CeA, but not in BLA, suggesting memory reconsolidation. Lastly, we showed the specificity of reconsolidation for the conditioning context, as demonstrated by the lack of rpS6P increase in a new context.
Our findings indicate that reconsolidation can occur for food instrumental memory, and propose the two-component molecular assessment with Zif-268/rpS6P as a reliable molecular assessment for instrumental retrieval and reconsolidation, in spite of no-behavioural output at behavioural tests.

Analysis of Satb2 expression and its function in IEG regulation in Visual Cortex

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PhD programme
Signal Processing in Neurons (SPIN)

Satb2 is well expressed in the pyramidal neurons of the cerebral cortex but its role in the plasticity of the mature cortex is unknown. Previously we have shown that Satb2 is necessary for L-LTP and affects Arc expression in CA1. We also show that Satb2 is induced by BDNF and activity in primary hippocampal neurons. These findings point to a function of Satb2 in homeostatic plasticity. In this study, we aim to characterize the expression and functions of Satb2 in visual cortex, a well-studied part of cortex for plasticity.
Analysis of Satb2 expression showed upper layers have higher percentage of Satb2+ neurons. Among Satb2+ neurons, layer5 neurons have highest Satb2 concentration. Measuring co-expression of cortical layer determinants (Satb2, Ctip2 and Tbr1) revealed that, with respect to combinations, upper layers are homogenous while lower layers are heterogeneous.
Dark rearing adult mice for 4d revealed a small but significant effect of neuronal activity on Satb2 levels. Because Satb2 levels were measured as relative levels between layers the direction of regulation could not be assessed. During the initial assessment, Satb2 expression was found to vary between neurons. Also, Satb2 concentration does not correlate with nuclear volume. These findings provide circumstantial evidence for possible regulation of Satb2 levels.
We found a positive correlation between Satb2 and Egr1 levels in conditions of home cage, dark adapted and dark adapted and re-exposed to 2h of light in upper layers where Satb2 expression was homogenous. Layer6 showed a positive correlation after dark adaption, negative correlation when re-exposed to light and no correlation in home cage. This is because in layer6 Satb2 levels could distinguish two pyramidal neuron populations that are functionally distinct with respect to Egr1 response. To confirm that Satb2 is necessary for Egr1 expression we find that cKO mice have lower home cage Egr1 levels.
Further experiments will explore how Satb2 expression evolves during postnatal brain development, especially during eye opening and critical period. A time series of IEG response (Egr1, Arc and cFos) after 4d dark rearing comparing cKOs to controls is currently underway.

Poster # 113

Pharmacology and Neuroscience

Patient-derived glial precursor cell therapy for vanishing white matter disease

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Post-Doc

Vanishing white matter disease (VWMD) is a genetic leukoencephalopathy caused by mutations in any of the genes encoding the five subunits of the eukaryotic translation initiation factor 2B (eIF2B). VWMD leads to a failure of proper white matter development and myelination. Both, astrocytes and oligodendrocytes seem to be affected. As of today, there is no cure for the disease and patients die within a few years after disease onset.

Cell based therapy would represent a new approach in VWMD treatment. In this study we aim to generate induced neural stem cells (iNSCs) from patient-derived fibroblasts. To achieve this, we currently apply a previously published Sendai virus based protocol. Single point mutations in the eIF2B gene of generated iNSCs will be genetically corrected through CRISPR/Cas9 technology. Currently, we are testing this technology in previously generated (unaffected) control iNSCs. We are using a lentiviral construct mediating the expression of green fluorescent protein (GFP) and a mutated red fluorescent protein (RFP). After transduction of control iNSCs, mutated RFP is genetically corrected by CRISPR/Cas9 system. Optimized protocols obtained from these experiments will be applied for genetic correction of patient-derived iNSCs.

Genome-edited iNSCs as well as control iNSCs will be differentiated towards the glial fate. Oligodendroglial precursor cells (OPCs) would represent a valid cell population for later transplantation studies. A prerequisite of OPCs is their high proliferation rate, high migratory capacity and their differentiation potential into both, astrocytes and oligodendrocytes. In a proof-of-principle study, OPCs will be transplanted into a mouse model of VMWD. Successfully transplanted OPCs are expected to migrate, differentiate and integrate into the brain, thereby replacing astrocytes and oligodendrocytes affected in VWMD.

This study represents the first study aiming to transplant genome-edited OPCs into the brain of a mouse model of VWMD.

Interaction of two autism risk genes: L-type calcium channel Cav1.3 (CACNA1D) and transcription factor T-brain-1 (TBR1)

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PhD programme Pharmaceutical Sciences

T-brain 1 (TBR1) is a brain-specific T-box transcription factor that has been identified by human genetic studies as a high-confidence risk gene for autism spectrum disorders. Due to its importance during brain development, individuals with heterozygous deficiency show abnormal inter- and intraamygdalar projections and NMDA receptor hypoactivity which leads to excitation/inhibition imbalance in the brain and finally to autistic-like behaviors. TBR1 directly regulates promoter activity of various downstream target genes and only two direct interaction partners are known: CASK (enhances the transcriptional activity of TBR1) and FOXP2.

Preliminary data of our group from yeast-two hybrid studies indicate that TBR1 specifically interacts with the distal C-terminus of the L-type calcium channel Cav1.3 (CACNA1D). As gain of function mutations (e.g. A749G mutation) in the Cav1.3 channel also lead to autism spectrum disorders, we propose that the interaction of TBR1 with the Cav1.3 C-terminus defines a new signaling pathway involved in normal brain function.

The interaction of myc-tagged TBR1 with the Cav1.3 C-terminus was verified by GST-pulldowns. Myc-TBR1 interacts with both C-terminally long (Cav1.3L) and short (Cav1.343S, Cav1.342A) splice variants. Stronger interaction with Cav1.3L suggests a major contribution of the distal C-terminus for TBR1 binding. Additionally, preliminary fluorescence microscopy experiments show that when TBR1 and the long Cav1.3 C-terminus are co-expressed in tsA201 cells, TBR1 targets the Cav1.3 C-terminus to the nucleus. However, when co-expressing TBR1 and Cav1.3 C-terminus fused to a plexstrin homogy domain for plasma membrane targeting, no translocation of nuclear TBR1 to the plasma membrane was observed.

In further GST pulldown experiments we will confirm our previous yeast-two hybrid studies predicting specific interaction of TBR1 only with Cav1.3 but not with Cav1.2, Cav1.4 and Cav2.1 alpha1-subunits. Moreover, we will determine binding of native TBR1 from brain lysates to the Cav1.3 C-terminus in GST pulldown experiments and perform co-immunoprecipitation studies of Cav1.3 and TBR1 to verify this interaction in adult and/or embryonic brain. We will also test for the presence of proteolytically processed C-terminal Cav1.3 peptides which could affect transcription by binding to TBR1 in the nucleus.

Poster # 115

Pharmacology and Neuroscience

Light induced ganglion cell responses in Cav1.4 mutant mouse retinas

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PhD programme
Pharmaceutical Sciences

Cav1.4 L-type calcium channels (LTCC) are predominantly expressed in the retina where they are located at the synaptic sites of photoreceptors and most likely also bipolar cells. Cav1.4 channels are characterized by their rapid activation, the relative negative activation voltage the slow inactivation. These properties are necessary to sustain the calcium influx at the ribbon synapses to provide a high rate of neurotransmitter release. Mutations in the CACNA1F gene encoding for the alpha1 subunit of Cav1.4 channels are known to cause Congenital Stationary Night Blindness Type 2 (CSNB2). In heterologous expression system, Cav1.4 I745T (IT) has been shown to cause a gain-of-function mutation. How such abnormal calcium influx can affect the retinal circuits is hardly known. Our previous work has demonstrated that the IT mutation caused disturbances in the signal transmission of mouse retinas using multielectrode array recordings upon visual stimulation in mesopic conditions. The aim of the current study is to further examine the ganglion cell (GC) activity of IT mouse retinas under both dim light (scotopic) and bright light (photopic) conditions, and by the mean of multiple light stimuli aimed to detect specific GC response pattern. Our preliminary results confirm a higher spontaneous firing rate in the absence of stimuli and a delayed response in both light conditions in IT whole-mount retinal preparations. Also, compared to controls, IT retinas showed a diminished firing frequency within the stimulus. Under mesopic light conditions many GCs did not respond to full-field stimulation, whereas in this study, the analysis of the same cell in two different light conditions showed that IT GC´s ON and OFF responses are largely lost during bright light but not using dim light stimuli. These preliminary data might indicate that in the IT CSNB2 model the cone pathway might be more severely affected; similar to what is seen in electroretinograms of CSNB2 patients.

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Molecular Cell Biology and Oncology (MCBO)

Signal Processing in Neurons (SPIN)

Host Response in Opportunistic Infections (HOROS)

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