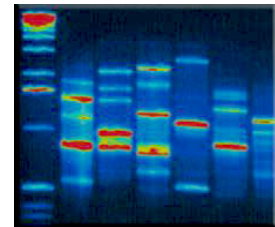
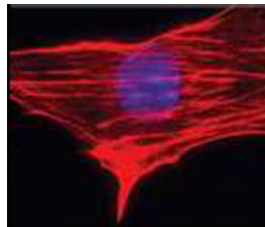




2nd Meeting of Doctoral Students

November 10th 2006 Innsbruck Austria



PROGRAM
AND
ABSTRACT BOOK

Foreword of the Students Representatives

It is our main goal to generate a platform for scientific discussion and cooperation between students belonging to different doctoral programs within MUI. We intend to gather as many students as possible, giving them a chance to present themselves and their work, to peers and professors.

For the professors, this is a chance to take a closer look at the “new generation”, to get a broad view of the science and scientists our University is producing.

We wish all of you interesting posters, fruitful discussions and time for personal contacts during the meeting, breaks and at the party.

Mag. Simon Schnaiter

(Chairman of the Students
Representatives)

Mag. Marco Rupprich

(1st Vice-chairman of the Students
Representatives)

Mag. Mariana Eca Guimaraes de Araujo

(2nd Vice chairman of the Students Representatives)

Program

2nd MEETING OF DOCTORAL STUDENTS

Time: Friday, November, 10th, 2006, 11:00 - Open end

Location: Chirurgie Hauptgebäude

Lecture Hall (1st floor)

Anichstraße 35

A-6020 Innsbruck

Local Organizer: ÖH doctoral students' representatives

Email: PhD@i-med.ac.at

Homepage: www.i-med.ac.at/oeh/phd

Program	
11:00	Open door for hanging posters (small lecture hall)
13:00 -14:15	1 st Poster Session (small lecture hall)
14:15-14:30	Coffee break
14:30-15:45	2 nd Poster Session (small lecture hall)
15:45-17:00	Refreshments and discussion
17:00-17:15	Doctoral programs at Innsbruck's Medical University A. Univ-Prof. Dr. Bernhard Flucher (big lecture hall)
17:15 – 18:15	SFB021 Lecture Univ.-Prof. Dr. Josef Penninger IMBA. (big lecture hall)
18:15...	Prizes announcement and open end Party (big lecture hall)

- **Abstract submission for poster presentation:**

Students participating in one of the doctoral programs at Innsbruck Medical University are invited to submit an abstract for poster presentation (max. 200 words abstract body; 1,5 line spacing; Arial font size 12; justified). **Abstract MUST be written in English!!!**

Submission should include: title of poster; author(s); affiliation(s); abstract and the name of the doctoral program in which the student is enrolled. Please note that **the student must be the main author of the work presented.**

Abstracts should be submitted to the following email address: PhD@i-med.ac.at

Deadline for abstract submission is the 18th October

By 25th October, authors will be informed by email, if their abstract has been selected for poster presentation. Poster size should not exceed A0 format (upright).

- **Poster session rules:**

Authors are expected to stand in front of their posters during their Poster session. Posters with even numbers will be presented on session 1. Posters with odd numbers will be presented on session 2.

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We would like to thank the following companies and persons for the financial support of the meeting:

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- Dr. Martin Köllensperger
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- Mag. Daniela Weiskopf
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- Mag. Daniela Ortner
(Associated Member)

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Doctoral Program

The Aging of Biological Communication Systems

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2. Cytomegalovirus-specific CD8⁺ T cells suppress the cellular response to Influenza in elderly persons

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Background: The immune response to Influenza is typically decreased in elderly persons which leads to a severe course of disease and death. Not only aging but also latent cytomegalovirus (CMV) infection, which leads to premature immune senescence, may contribute to their unresponsiveness to influenza infection.

Methods / Results: We now show that elderly persons who are chronically infected with CMV have a higher frequency of CMV-specific CD8⁺ T cells whereas their fraction of Influenza (FLU)-specific CD8⁺ T cells is significantly smaller compared to young persons ($p < 0,01$). During in vitro co-stimulation of purified CD8⁺ T cells from 8 elderly (>60 years) and 6 young (<35 years) persons with the Influenza M1₅₈₋₆₆ peptide and the CMV_{pp65} peptide, the propagation of FLU-specific CD8⁺ T cells is inhibited by the expansion of CMV-specific CD8⁺ T cell clones. This dominance of CMV-specific CD8⁺ T cells is not due to higher precursor numbers, but due to a higher affinity of their T cell receptors (TCR) to the antigenic peptide/MHC complex. CMV-specific CD8⁺ T cells respond to lower peptide concentrations and have an increased capacity to bind peptide/MHC multimers than corresponding FLU-specific CD8⁺ T cells. They also show a limited clonal diversity and use a very restricted set of TCR V-beta chains, indicating a preferential growth capacity of a few selected clones.

Conclusion: We conclude that latent CMV infection represents a risk factor for elderly persons to have a diminished immune response to Influenza.

3. Cadmium a novel risk factor for arteriosclerosis? Mechanisms and *in vivo* Relevance

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Abstract:

Cigarette smoke is one of the most important risk factors for cardiovascular diseases (CVD). However, the underlying mechanisms are currently not understood. Among approximately 4800 cigarette smoke chemicals our previous analyses suggest, that CS-oxidants and metals are crucial for the CVD causing activity of CS.

The present project is aimed to investigate the *in vitro* effect of Cadmium (Cd) which is increased in the serum of young smokers. Since previous microarray analyses of primary endothelial cells (ECs) exposed to Cd suggest, that Cd affects the fundamental structural and adhesion components of ECs, we recently performed a detailed RT-PCR-based-analysis of structure-relevant genes. In detail Vimentin, VE-Cadherin, β -Catenin and hsp27 were tested.

These experiments show a downregulation of structural and adhesion molecules after incubation with Cd for 24 hours. In order to test whether the altered gene expression induced by Cd has consequences for the functionality of the vascular endothelium, ECs were grown on a permeable membrane and incubated with various concentrations of Cd for 24 hours. In response to Cd presence in the cultures the integrity of the monolayer was significantly destroyed. In conclusion, the data presented support the hypothesis that Cd is a novel independent risk factor for arteriosclerosis.

4. CD25-Expressing, non-regulatory CD8⁺ T lymphocytes occur in healthy elderly persons and represent a repertoire of diversity

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¹Institute for Biomedical Aging Research, Austrian Academy of Sciences, Innsbruck, Austria, ²Institute for Legal Medicine, Innsbruck Medical University, Innsbruck, Austria.

Background: Infectious diseases are frequent and severe in elderly persons and the efficacy of vaccinations is low. This is due to an age-related decline in the functions of the immune system referred to as immunosenescence. Recently, we identified an interleukin (IL)-2 / IL-4-producing CD8⁺CD25⁺ non-regulatory memory T cell population that occurs in a subgroup of healthy elderly persons who characteristically still have a good humoral immune response after influenza vaccination.

Methods: FACS, flow FISH, CDR3 spectratyping, molecular tracking of clones, intracellular cytokine staining, microarray technology and quantitative RT-PCR were used to analyze the memory CD8⁺ T cell subsets from persons over 60 years of age.

Results: We demonstrate that CD8⁺CD25⁺ memory T cells have a characteristic gene expression profile, longer telomeres and display a highly diverse TCR repertoire compared with their CD8⁺CD25⁻ memory counterparts. Molecular tracking of specific clones revealed that the same clones occur in both, CD8⁺CD25⁺ and CD8⁺CD25⁻ memory T cells, demonstrating a lineage relationship between CD25⁺ and CD25⁻ memory CD8⁺ T cells. This is further supported by long-term antigenic challenge, as CD8⁺CD25⁺ memory cells acquire characteristics of CD8⁺CD25⁻ cells.

Conclusion: The accumulation of CD8⁺CD25⁺ memory T cells in a subgroup of elderly persons appears to be a prerequisite of intact immune responsiveness in the absence of naive T cells in old age.

5. Regulation and Function of GAGEC1 – Implications for Prostate Cancer and Benign Prostatic Hyperplasia?

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Background: Prostate cancer (PCa) and benign prostatic hyperplasia (BPH) are two of the most common diseases of the aging male. We established an *in-vitro* model for BPH and identified GAGEC1 as a candidate gene for prostate tissue-remodeling. The expression of GAGEC1, a member of cancer/testis-associated antigens, is restricted to reproductive tissues and upregulated in prostatic diseases. As in age TGF- β and steroid hormone production changes and their role in the onset and progression of BPH and PCa we investigated their regulation of GAGEC1.

Methods: Prostate tumor cell-lines were treated with oestrogen [10^{-7} M], dihydrotestosterone [10^{-7} M], 5-aza-2`deoxycytidine [0,5 μ M] and sodiumbutyrate [0,5mM]. After definite time periods RNA was isolated, reverse transcribed and used for relative quantification (qPCR).

Results: Bioinformatic analysis from the GAGEC1-promoter region indicated predicted consensus transcription factor binding sites for several steroid hormone receptors, TGF- β -signalling and prostate-specific transcription factors (e.g. NKX3.1). In addition to regulation by TGF- β 1 an inductive effect by androgen and oestrogen was shown on GAGEC1 gene expression in a time-dependent manner as did 5-aza-2`deoxycytidine and sodium butyrate, chromatin-remodeling agents, in several different tumour cell-lines tested. This effect was not observed in primary prostatic and endometrial stromal fibroblasts.

Conclusion: Given the association of increased expression with prostatic disease and its hormonal regulation, GAGEC1 may represent a diagnostic molecular marker and therapeutic target for the treatment of BPH and PCa.

Supported by Austrian Science Fund (FWF; NRNS907-B05),

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6. Mesenchymal stem cell aging

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Background: Mesenchymal stem cells (MSC) appear to be located throughout the adult body of higher organisms, thereby supporting a continuous renewal and repair of various tissues. However, by now it is poorly understood how MSC are involved in tissue maintenance during aging, nor is it known how MSC are influenced by their microenvironment.

Methods: In order to study the changes MSC undergo during in vitro and in vivo aging, they were derived from bone marrow of both females and males of different ages, cultivated, and examined considering the following stem cell properties: Proliferative capacity in vitro as an indicator for self-renewal capacity, colony formation of stem cells in primary cultures as an indicator for the quantity of MSC in vivo, fluid phase endocytosis as an indicator for the communication with the stem cell's microenvironment, telomere length, and mRNA levels of several candidate aging biomarkers revealed by array analysis.

Results: With donor age, proliferative capacity in vitro and number of MSC in vivo seem to decrease, whereas telomere length and endocytosis activity do not change. However, endocytosis activity increases when cells become senescent, and VCAM-1 mRNA levels increase with donor age.

Conclusion: The results indicate that endocytosis activity is a marker for in vitro senescence, and VCAM-1 is a marker for in vivo aging of MSC.

Doctoral Program

Regulation of Gene Expression During Growth, Development and Differentiation

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7. Protein patterns of sporulation stages of *Physarum polycephalum*

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The multinuclear slime mold *Physarum polycephalum* is a model organism for studying cell cycle and differentiation. The transformation from plasmodium to sporangia is a dramatic differentiation processes. The organism needs a starvation period to gain sporulation competence. This period is known to be accompanied by induction of nitric oxide synthases. After starvation, a light pulse triggers the sporulation process. Here we compare protein patterns of cultures with and without light pulse, and throughout the starvation period. Cultures were divided in two equal halves. One was collected for protein analysis, the other was further incubated to control sporulation behaviour. Protein extracts to be compared were labelled with different fluorescent dyes, mixed, separated together by two-dimensional gel electrophoresis and analysed by three colour fluorescence imaging. On average, we could resolve about 2300 matched protein spots per gel. The threshold for expression difference was set to 2-fold, significance of difference was judged by Student's T-test. The light pulse leads to up-regulation of 6 spots, and down regulation of 49 spots. Starvation up-regulates 7 spots and down-regulates 72 spots. From the sequence of these spots we hope to get a more comprehensive picture of proteins involved in the differentiation process of *Physarum polycephalum*.

8. A novel mechanism of regulation of iron-dependent pathways in eukaryotes is mediated by HapX interacting with the CCAATbinding complex

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²Division of Biological Chemistry, Biocenter, Innsbruck Medical University, Innsbruck, Austria.

Background: Subtle control systems are required to maintain iron homeostasis. In *Aspergillus nidulans* iron represses iron acquisition via the GATA-type transcription factor SreA and induces iron-dependent pathways by a so far unknown mechanism, the characterization of which was the aim of this work.

Methods: Comparison of growth phenotypes, transcriptional iron regulation, siderophore production, and heme biosynthesis in mutants lacking HapX, the CCAAT binding complex (CBC) subunit HapC or SreA.

Results: HapX represses expression of iron-dependent proteins and pathways (e.g. aconitase, and heme biosynthesis) during iron depleted conditions by physical interaction with the CBC. In contrast, extracellular siderophore biosynthesis is activated by CBC-HapX interaction. Mutual transcriptional control of HapX and SreA and synthetic lethality of deletion of both regulators suggests a tight interplay of these two iron homeostatic control systems. Growth phenotypes indicate that the CBC has a general role independent of the iron status, whereas HapX function is confined to iron depletion. Consistently, expression of HapX is repressed by iron, whereas that of HapC is constitutive.

Conclusion: Our study describes a novel iron regulatory mechanism in eukaryotes. These finding might also be important in the development of novel antifungal therapies as fungal iron-homeostatic mechanisms are crucial for virulence. Doctoral program: Regulation of gene expression during growth, development and differentiation

9. **AbcB, an iron-regulated ABC transporter of *Aspergillus fumigatus* involved in excretion of fusarinines**

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Background: *Aspergillus fumigatus* represents an important opportunistic pathogen in immunocompromised patients. Siderophore biosynthesis is essential for its virulence. The fungus produces two siderophores: it excretes desferri-triacetylfusarinine C to mobilize extracellular iron and accumulates desferri-ferricrocin for iron storage. Subsequent to uptake, triacetylfusarinine C is hydrolyzed, and the resulting fusarinines are excreted. The *A. fumigatus* ABC transporter-encoding *abcB* was found within an iron-regulated gene cluster, indicating a role in iron metabolism.

Methods: AbcB was functionally characterized by generation of a respective deletion mutant, analysis of its siderophore production and growth rate, GFP fusion-mediated subcellular localization and phylogenetic analysis.

Results: AbcB is localized in the plasma membrane. Its deletion causes increased intra- and decreased extracellular fusarinine accumulation, and impaired growth rate during iron depletion. AbcB is the prototype of an ABC transporter superfamily clade of so far unknown function, with members in siderophore-producing but not siderophore-lacking species. Our results implicate a role in siderophore metabolism for all members of this clade.

Conclusion: Most members of the ABC transporter superfamily are implicated in multidrug resistance. AbcB is involved in fusarinine excretion and therefore one of few ABC transporters, and the first of *Aspergillus*, with a known physiologically relevant function in the absence of xenobiotics.

10. The retinoblastoma related pRb2/p130 is functional regulated by acetylation.

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Background: p130, an Rb-related protein and member of the pocket protein family, exerts regulatory functions during the cell cycle. Its functional activity is regulated by phosphorylation.

Methods: Nuclear extracts of synchronized NIH-3T3 cells were analyzed for the presence of modified subspecies of p130, especially acetylated p130, by chromatography and immunoprecipitation. Truncated versions of the protein were overexpressed using an insect-cell system, subjected to in-vitro acetylation with p300 and analyzed using autoradiography and mass spectrometry.

Results: Acetylated and hyperphosphorylated p130 coeluted in fractions after anion exchange and size exclusion chromatography. Acetylated and hyperphosphorylated p130 could be immunoprecipitated from S/G2 synchronized NIH-3T3 cells. In vitro assays using p300 as the acetyl-transferase showed major acetylation in the C-terminus of p130, mass spectrometry pinpointed the major acetylation site K1079 located within a nuclear localization sequence.

Conclusion: We present evidence that the regulation of p130 is not only achieved by phosphorylation and cellular localization but also by acetylation. Our data show cell cycle dependent acetylation in vivo. Furthermore, p300 is able to acetylate p130 invitro with the major acetylation site in an NLS in the C-terminus of the protein.

11. Functional analysis of protein arginine methyltransferases in *Aspergillus nidulans*.

I. Bauer, S. Graessle, P. Loidl, G. Brosch

Division of Molecular Biology, Biocenter, Medical University, Innsbruck, Austria

Background: *Aspergillus nidulans* harbors three genes encoding protein arginine methyltransferases (PRMTs), namely *rmtA*, *rmtB*, and *rmtC*. Functions of PRMTs, mainly demonstrated for higher eukaryotes, implicate a role in transcriptional regulation, RNA processing, signal transduction, and DNA repair. Since filamentous fungi are important in biotechnology but also are producers of toxins (e.g. aflatoxin) and cause human diseases, we use the ascomycete *A. nidulans* to elucidate the function of PRMTs in this model system.

Methods: We generated PRMT deletion strains which were analyzed under different growth conditions. For biochemical characterization, proteins of fungal extracts were fractionated by chromatography and subjected to methyltransferase activity assays, fluorography, and immunoblot analysis.

Results: All strains are viable and grow well under standard growth conditions. However, $\Delta rmtA$ and $\Delta rmtC$ exhibit higher susceptibility to H₂O₂. Biochemical characterization of *wt* and mutant strains revealed that *A. nidulans* PRMTs can be separated by anion-exchange chromatography, and exhibit methyltransferase activity corresponding to the reduced total activity in deletion strains.

Conclusion: Unlike RmtB, RmtA and RmtC are important for oxidative stress tolerance, which is a prerequisite for survival of pathogenic fungi in their host organisms. In case methylation activity is essential for this function, the next aim will be to isolate substrate proteins involved in oxidative stress response.

12. Enoyl-CoA hydratase-encoding *sidH* is essential for biosynthesis of the extracellular siderophore desferri-triacetylfusarinine C of *Aspergillus fumigatus*

M. Gründlinger, M. Schrettl and H. Haas

Division of Molecular Biology/Biocenter, Innsbruck Medical University, Fritz-Pregl-Str. 3, A-6020 Innsbruck, Austria

Background: Virtually all organisms require iron as an essential nutrient. Siderophores, low molecular mass, iron-specific chelators, play a central role in iron acquisition and storage in most fungi. The opportunistic pathogen *Aspergillus fumigatus* produces two major siderophores: it excretes desferri-triacetylfusarinine C (DF-TAFC) to mobilize extracellular iron and ferricrocin for intracellular iron storage. The siderophore system, which is poorly characterized, represents a promising new target for antifungal drugs as it is essential for virulence of *A. fumigatus* but absent in mammalia.

Methods: Iron acquisition is regulated by the GATA-factor SreA. SreA-mediated upregulation of the *A. fumigatus* gene *sidH* during iron depleted conditions indicated involvement in iron metabolism. Functional characterization of SidH was carried out by generation of a respective loss-of-function mutant (*!sidH*) and its comparison to wild type with respect to growth rate and siderophore production.

Results: *!sidH* revealed wild type-like ferricrocin production but lack of DF-TAFC synthesis. Consistent with involvement in DF-TAFC production, *!sidH* showed a decreased radial growth rate under iron starvation.

Conclusion: The structure of DF-TAFC (a cyclic peptide consisting of three *N*₅-*cis*-anhydromevalonyl-*N*₅-hydroxy-L-ornithine residues linked by ester bonds) and the similarity of SidH to enoyl-CoA hydratases suggests that SidH catalyzes the conversion of mevalonyl-CoA to *cis*-anhydromevalonyl-CoA.

Doctoral Program

Image-guided Diagnosis and Therapy

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13. Holographic Laser Tweezers for flexible optical Micromanipulation

A. Jesacher, S. Fürhapter, C. Maurer, S. Bernet, and M. Ritsch-Marte

Division for Biomedical Physics, Innsbruck Medical University, Müllerstraße 44, A-6020

Innsbruck, Austria

Background: Optical tweezers are tools for manipulating and trapping microscopic specimens with light. The applied forces are in the range of 10^{-12} N and origin from the momentum of photons. Our aim in this area of research is to find new methods and fields of applications for optical tweezers.

Methods: Holographic optical tweezers (HOT) make use of a spatial light modulator (SLM) to shape the tweezers laser prior to coupling it into a microscope objective. Using HOT, we perform experiments on micro-beads located in an air-liquid interface, which naturally stabilizes the particles due to surface tension. As a consequence, they can be manipulated by a far greater “set” of light patterns, since only two-dimensional trapping within the interface has to be provided by the laser.

Results: We demonstrate different optical “micro tools”, which utilize scattering and gradient forces to enable controlled transport of matter, and show that we can in principle get access to the visco-elastic properties of the interface.

Conclusion: As a new working environment for HOT, an air-liquid interface enables the application of weakly focussed light fields with small axial field gradients. Consequently, also objectives with low numerical apertures can be utilized. In future, HOT may be stronger involved into the examination of surface properties at micro scale.

14. Functionalized Nanoparticles for high specific radiolabelling with diagnostic and therapeutic radionuclides.

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Background: Nanotechnological methods are considered a promising new tool in nuclear medicine for molecular imaging and therapy. Nanoparticles have been successfully derivatised on their surface for radiolabelling with either different functional groups, receptor-ligands or proteins. Due to high specific activity, different pharmacokinetics and potentially increased specific binding, a stronger signal can be achieved in imaging, and an increased therapeutic efficacy can be expected.

Methods: Various types of nanoparticles, based on albumin, protamin and lipids, derivatised with DTPA and DOTA, have been tested for radiolabelling with ^{99m}Tc and, ¹¹¹In for SPECT (single photon emission computed tomography), ⁶⁸Ga for PET (positron emission-tomography) and ¹⁷⁷Lu for therapeutic applications, optimizing the reaction conditions, such as reaction-time, pH and temperature. Labeling and stability in phosphate buffered saline, 4 mM DTPA and serum of labeled nanoparticles have been analyzed with ITLC and SEC *in vitro*.

Results: High labeling yields with different radionuclides were achieved exceeding a specific activity of 1GBq/μmol DTPA. Radiochemical purity resulted to be >95% for ¹¹¹In, ⁶⁸Ga and ¹⁷⁷Lu, whereas some types of NPs showed lower labeling yields with ^{99m}Tc. Radiolabelled NPs demonstrated high stability in phosphate buffered saline and against transchelation in 4mM DTPA. The stability in serum varied between the different types of NPs, the radiochemical purity measured 24h after incubations was always >50%.

Conclusion: The different types of NPs have been successfully labeled with high yield and high radiochemical purity with ¹¹¹In, ⁶⁸Ga and ¹⁷⁷Lu, labeling yields with ^{99m}Tc varied dependent on the nature of NPs. Sufficient stability has been shown in first *in vitro* examinations leading to further evaluation *in vivo* for oncological indications after fictionalization with adequate tumor specific ligands.

15. Multipolar Radiofrequency Ablation: Inducing Large Necroses in Ex Vivo Bovine Livers. An Experimental Study

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Background: Radiofrequency ablation (RFA) has the potential to irreversibly destroy tumorous tissue. Thereby high-frequency alternating current is delivered through needle-like applicators, resulting in ionic agitation and friction which leads to local heating around the applicator's tip. So far, a maximum of 3 bipolar applicators used simultaneously in a resistance controlled manner has been investigated in ex vivo studies to create large lesions (diameter > 3 cm) in a single application.

Methods: Up to 6 internally cooled bipolar applicators are combined to create lesions in fresh, explanted bovine livers. Applied energy as well as distance between the applicators are varied systematically. The size and shape of the lesions are determined via magnetic resonance imaging as well as macroscopically after section.

Results: The study is ongoing. First, a MR-sequence had to be found to achieve correct and well-contrasted imaging of the lesions. The T1 TSE-sequence shows best correlation of 3D-imaging and macroscopically measured necrosis. So far 50 ablations have been performed. The parameters governing the ablative process are recorded continually and will be used to summarize the results to a protocol for ablation of large liver tumors.

Conclusion: Applicator distance and amount of applied energy are key factors for creating large, confluent and regularly shaped lesions.

16. Application of a Spatial Light Modulator for Fourier plane filtering of microscopic samples

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Background: A spatial spiral phase filter located in the Fourier plane of the microscopic imaging pathway is used for edge-enhancement of microscopic amplitude and phase samples.

Methods: The phase filter is displayed at a reflective spatial light modulator that is used as a phase diffractive element. The method can be isotropic, i.e., all edges within the sample are highlighted simultaneously. Separate manipulation of the phase of the central area of the spiral phase filter leads to a symmetry break that results in a relief-like impression of the sample. This appearing shadow effect can be used for a quantitative reconstruction of the sample topography if at least 3 images of a sample with different shadow orientations are post-processed numerically.

Results: After a former calibration with a test sample the optical thickness of biological samples can be reconstructed with an accuracy of a few 10 nm.

Conclusion: A spiral phase filter can be used for an isotropic edge enhancement of microscopic samples. Separate control of the central area of the filter leads to the impression of a shadow effect. At least 3 images of a sample with different shadow orientations are needed for a quantitative reconstruction of the optical thickness.

26. Accuracy Comparison of Marching Cubes and Deformable Models for Hard-tissue Segmentation

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Background: The quality of surgical navigation relies on the accuracy of the registration, where the good segmentation comprises a substantial component. The study compares the accuracy of two segmentation methods: a grayvalue thresholding, followed by a triangulation with marching cubes, and a deformable model. Both are applied on CT-datasets to delineate the occipital bone, which is to be used for automatic segmentation-based registration in a further research effort.

Methods: The thresholding plus marching cubes segmentation (TMCS), and the deformable model segmentation (DMS) are compared in two ways: quantitatively, by overlaying the areas of the segmentations in cross-sectional slices, and visually, by displaying the pointwise distances between the segmentations in a color-coded graphic. Both segmentations are compared to an expert manual segmentation, taken as a „ground truth“. The progress of the deformation is displayed for preset parameters and fixed number of iterations. A separate plot shows the evolution of the mesh coordinates until convergence of the deformation algorithm.

Results: The results show, that in delineating clear contours in medical images, a TMCS is closer to a manual segmentation and more accurate for registration.

Conclusion: While in noisy images and soft tissue delineation, a gradient-based method, like the deformable model, would achieve a better fit to the real surface, the grayvalue interpolation, incorporated in marching cubes, better delineates hard-tissue with sub-voxel accuracy.

Doctoral Program

Infectious Diseases: Molecular Mechanisms

17. Complement evasion of *Candida* spp. and *Aspergillus* spp. by acquisition of fluid-phase inhibitors

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Candida spp. and *Aspergillus* spp. represent major causes of severe, and often lethal, systemic opportunistic fungal infections in immunocompromised hosts. In host defense against fungi complement plays an important supportive role. Fungi, however, aim to evade complement action. The aim of the study was to investigate complement evasion by *Candida glabrata*, *Candida dubliniensis*, *Aspergillus fumigatus* and *Aspergillus terreus* by assessing their properties to bind FH and C4bp, the main fluid phase regulators of complement.

Fungi were incubated with human EDTA-plasma or C4bp at 4°C, followed by fixation and blocking. Primary antibodies were detected via secondary FITC-labelled antibodies. For immunofluorescence cells were counterstained with EvansBlue®. In parallel cells were also examined by FACS, 10.000 events were routinely counted.

All *Candida* and *Aspergillus* strains investigated bind both the alternative pathway inhibitors factor H and FHL-1 and the classical pathway inhibitor C4bp as assessed by immunofluorescence and FACS studies.

In conclusion, *Candida dubliniensis*, *Candida glabrata*, *Aspergillus fumigatus* and *Aspergillus terreus* bind to fluid phase inhibitors FH, FHL-1 and C4bp. Binding of these inhibitors to *Aspergillus* spp. appears to be stronger than to *Candida* spp. Employment of complement inhibitors by pathogens results in down-regulation of complement activation, as previously shown for *Candida* spp.

Doctoral Program

Molecular Cell Biology

physiologie.uibk.ac.at/mcb

18. Zebrafish as model organism for studying skeletal muscle ECC: Expression of exogenous calcium channel β_{1a} subunit can completely restore the motile phenotype in paralyzed β_{1a} -null zebrafish

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Skeletal muscle excitation-contraction coupling (ECC) depends on protein-protein interaction between the plasmalemmal voltage-gated $\text{Ca}_v1.1$ and the sarcoplasmic reticulum Ca^{2+} release channel (RyR). This mechanical coupling requires the coordinated arrangements of $\text{Ca}_v1.1$ in groups of four opposite the RyR (tetrads). The immotile zebrafish strain *relaxed* (red^{t25}) lacks tetrad formation and thus ECC due to $\text{Ca}_v1.1$ β_{1a} subunit deficiency. To test β_{1a} -null as the monogenetic trait of the *relaxed* phenotype, in-vitro synthesized β_{1a} RNA was injected into zebrafish zygotes for putative rescue of the motile phenotype in the larvae. Isolated *relaxed* muscle cells were transfected with β_{1a} and analyzed by fluorescence immunocytochemistry and by electrophysiology (whole-cell patch clamp). Recovery of tetrad formation was analyzed by freeze-fracture electron microscopy of fixed tail muscle tissue.

In-vivo, injection of β_{1a} RNA was able to transiently recover normal larval motility. Exogenous β_{1a} was correctly colocalized into triadic clusters with all other $\text{Ca}_v1.1$ subunits and RyR and restored the ultrastructural arrangement of $\text{Ca}_v1.1$ in tetrads. All biophysical parameters of skeletal-type ECC were indistinguishable from wild type.

Quantitative *in-vivo* and *in-vitro* rescue of ECC shows that zebrafish *relaxed* can serve as a very potent model organism for studying the structural-functional role of β_{1a} for skeletal muscle-type ECC using β isoforms and chimeras.

19. CSNB2 mutation K1591X unmasks calcium-dependent inactivation (CDI) in Cav1.4 Ca²⁺ channels

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Tonic neurotransmitter release at retinal synapses is mediated by Ca²⁺ influx through slowly inactivating Ca_v1.4 L-type voltage-gated Ca²⁺ channels (LTCCs) lacking Ca²⁺-dependent inactivation (CDI). Ca_v1.4 α 1 subunit mutations cause human congenital stationary night blindness type-2 (CSNB2). Here we report the functional properties of K1591X (KX), a C-terminal truncation mutant after expression in HEK-293 cells (+ β 3+ α 2 δ 1). In whole-cell patch-clamp recordings KX supported I_{Ba} and I_{Ca} with densities comparable to WT but significantly shifted the voltage for half-maximal activation ($V_{0.5act}$) to more negative potentials. In contrast to WT, KX showed robust calmodulin (CaM)-dependent CDI. Removal of the last 55 or 122 (C₁₂₂) C-terminal amino acid residues restored CaM-dependent CDI and shifted $V_{0.5act}$ of the corresponding truncation mutant to more negative potentials (shift I_{Ba}: -9.7; I_{Ca}: -9.5 mV). Gating changes were reversed by co-expression of C₁₂₂. FRET experiments combined with electrophysiological analysis revealed the existence of a novel gating modulator residing within the last 122 residues of the Ca_v1.4 C-terminal tail inhibiting CDI by interacting with more proximal tail regions. This represents a novel mechanism for the modulation of CDI in LTCCs and explains the physiologically relevant absence of CDI in retinal neurons. Our findings also reveal a novel loss-of-function mechanism for human CSNB2.

Support: FWF (P-17159)

20. Interleukin-6 Exerts Different Effects on Proliferation and Signal Transduction in Two Androgen-Sensitive Prostate Cancer Cell Lines

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Background: Interleukin-6 (IL-6) is a multifunctional cytokine involved in regulation of proliferation, apoptosis, and angiogenesis in several cancers including prostate. Previous studies demonstrated that IL-6 can cause ligand-independent activation of androgen receptor (AR). Since the AR is expressed in most advanced prostate cancers, we have investigated regulation of proliferation and IL-6 signaling in two AR-positive cell lines representing advanced prostate cancer.

Methods: Proliferation of AR-expressing cells LAPC-4 and MDAPCa2b exposed to IL-6 was studied by ³H-thymidine incorporation assay. Expression and phosphorylation of STAT3, p44/p42, and Akt were analyzed by Western Blot. IL-6 mRNA and protein expression were investigated by RT PCR and ELISA, respectively.

Results: Statistically significant inhibition of LAPC-4 and stimulation of MDAPCa2b growth by IL-6 were observed. IL-6 did not cause phosphorylation of STAT3 in either cell line. Phosphorylation of p44/p42 by IL-6 was induced only in MDAPCa2b cells. Treatment of LAPC-4 with IL-6 yielded diminished phosphorylation of Akt. IL-6 expression was undetectable in both cell lines.

Conclusion: In the present study, we provide experimental evidence that IL-6 plays a role in the induction and inhibition of growth of AR-positive prostate cancer cells. Both cell lines could be a good model for studies on hormone independent prostate cancer.

21. IFN α induced proximal tubular cell toxicity involves an apoptotic death pathway.

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Background: Side effects of interferon therapy are a major problem in treatment of many neoplastic and viral diseases. Besides common flu-like symptoms major organ failures, such as acute renal failure, were documented.

Methods: In order to investigate the molecular mechanisms involved in IFN α induced nephrotoxicity we treated proximal tubular LLC-PK₁ cells with IFN α .

Results: Concomitant with the appearance of DNA condensation a hypodiploid cell population and Caspase-3, -8, and -9 enzymatic activities were detected in IFN α treated monolayers. Inhibition of mitochondrial permeability transition by Cyclosporine A reduced IFN α induced Caspase-3 activation. At high concentration Cyclosporine A, however, exacerbated IFN α induced apoptotic cell death. Similarly, proliferating LLC-PK₁ cell cultures displayed an increased susceptibility for IFN α induced apoptosis. Since proximal tubular cells are able to proliferate in response to a toxic or ischemic insult in vivo in order to replace dead cells, this result suggests that IFN α induced apoptosis might preferentially affect proximal tubular cells in recovery.

Conclusion: In conclusion, IFN α was shown to induce apoptosis of proximal tubular cells providing an explanation for renal function impairment by IFN α therapy. The reported in vitro data suggest that the nephrotoxic potential of IFN α might be significantly increased on pre-damaged kidneys.

22. Phosphoproteomics in MAPK-signaling

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Background: Scaffold proteins are regulators for signaling. One of the mammalian scaffold proteins for the MAPK pathway is MP1. The adaptor protein p14 localizes the MP1-MAPK-complex to late endosomes. Various results suggest that the subcellular compartmentalisation of signaling complexes shapes the signaling output. We aim to identify targets of the MAPK signaling cascade with a special focus on the phosphoproteome of the p14/MP1-signaling complex.

Methods and Results: For substrate screens, we use knock out cells missing one member of the signaling cascade. We use recombinant kinases and scaffold or adaptor proteins to rebuild signaling complexes in different composition. By reconstituting the complexes *in vitro* as well as *in vivo*, we plan to identify targets that are specifically activated depending on certain signaling-complex compositions.

In cooperation with partners in the Austrian Proteomics Platform, we develop strategies for the enrichment of phosphopeptides out of complex protein mixtures. In first experiments we performed kinase assays, incubating fractions of MAPK knock-out cells with activated MAPK in the presence of ATP. Tryptic phosphopeptides were enriched by IMAC and analysed by MS. Several thousand phosphopeptides were measured. The list of identified proteins contained many low abundant proteins, known MAPK-targets and new interesting proteins.

Kinase assays in complex cell extracts show a high unspecific background of phosphorylation. To increase specificity, we engineer kinases mutated in the ATP-binding site allowing the use of orthogonal ATP-analogs that cannot be used by other wild type cellular kinases.

Conclusion: We plan to combine the reconstitution of signaling-complexes in knock-out cells, the use of ATP-pocket mutated kinases and the analysis of phosphorylated targets with sensitive chromatography – MS-based techniques to get a deeper insight into the phosphoproteome of MAPK-signaling.

23. PKCtheta selectively controls the adhesion-stimulating molecule Rap1 via direct RapGEF2 phosphorylation in T lymphocytes

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Background: A productive interaction of a T cell and an antigen-presenting cell (APC) results in the formation of an immunological synapse, a crucial step in promoting T cell activation. Beta2-integrins on the T cell, namely LFA-1, and its counter ligands, namely ICAM-1 on the APC, promote and stabilize antigen-specific interactions between these cells. The small GTPase Rap1A is essential for the critical LFA-1 /ICAM-1-mediated adhesion, however, its regulatory components have not been resolved.

Methods: We investigate ICAM-1 adhesion signaling of T cells by employing transfection analysis, yeast-two-hybrid screens and Co-IPs, phosphostatus analysis, knock out as well as siRNA-knockdown approaches.

Results: We identify a previously unknown function of a PKCtheta-RapGEF2 complex for LFA-1 avidity regulation. Direct phosphorylation at Ser-960 on RapGEF2 by PKCtheta is regulating Rap1 activation as well as LFA-1 adhesiveness to ICAM-1. Gene ablation of either of the genes encoding PKCtheta, RapGEF2 and Rap1A impairs cellular adhesiveness of CD3+ T cells through LFA-1.

Summary: Our study demonstrates for the first time a critical and non-redundant role for PKCtheta and its substrate RapGEF2 in the T cell signaling to Rap1 to set the threshold for an effective and sustained immune response *in vivo*.

24. The C-terminal AKAP interaction domain is not necessary for normal expression and targeting CaV1.2 in hippocampal neurons

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Differential targeting of calcium channels in neurons is essential for their specific functions in neuronal physiology. In *Xenopus* oocytes membrane expression of heterologous CaV1.2 calcium channels is enhanced by coexpression of AKAP79 (Altier et al., 2002). We hypothesized that an intramolecular interaction between the C-terminus and the II-III loop causes the retention of the channel in the ER, and that binding of AKAP79 to a C-terminal leucine-zipper motif removes this inhibitory interaction, thus promoting membrane expression. Here we tested whether this mechanism is necessary for membrane expression and targeting of CaV1.2 in vivo. Cultured mouse hippocampal neurons were transfected with wild type and mutant CaV1.2 carrying an extracellular HA tag and membrane expression patterns were analyzed using live cell immunofluorescence. Wild type CaV1.2-HA was expressed in clusters along dendritic shafts and spines. Unexpectedly, site directed mutagenesis of the leucine-zipper did not change membrane expression and targeting of the channel. Thus, in neurons the C-terminal leucine-zipper is not essential for membrane expression and targeting of L-type calcium channels. Either AKAP79/150 binds to another domain or redundant targeting mechanisms in vivo make the contribution of AKAP79/150 obsolete.

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25. Life/death decisions in growth factor signaling: critical role for RAF and Bcl-2 proteins (MCBO)

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Background: Lack of sufficient growth factors is a common stimulus for the induction of apoptosis that can be suppressed by RAF, AKT or Bcl-2 proteins. It is currently unclear, which events translate the lack of survival signal into a death stimulus, and how they are controlled by survival proteins.

Methods: IL-3 dependent parental 32D cells or 32D cells expressing activated versions of RAF or PKB were used in growth factor abrogation experiments. Alteration in mitochondrial Ca^{2+} and ROS levels were monitored by confocal imaging following loading of cells with Rhod-2 or MitoSOX™ Red, respectively. Protein expression was verified by immunoblotting after SDS-PAGE.

Results: We show that following IL-3 withdrawal reactive oxygen species (ROS)-induced mitochondrial Ca^{2+} overload functions as an RAF-suppressible apoptosis trigger, while others demonstrated the requirement to inactivate Mcl-1 via an AKT-dependent pathway. Our results also suggest that Mcl-1 stability is not affected by RAF-signaling, while activated PKB, which we established as an effector of RAF in these cells before, suppressed mitochondrial ROS production and Ca^{2+} overload.

Conclusion: Life/death decisions following growth factors signaling may hinge on cooperative decision making by RAF-and Bcl-2 dependent signals.

27. Expression profiling of voltage-gated calcium channel subunits in mouse hippocampus and cultured hippocampal neurons using quantitative Taqman RT-PCR (MCBO)

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The importance and diversity of calcium signaling in the central nervous system is mirrored by the expression of a multitude of calcium channel types. Whereas the general patterns of voltage-gated calcium channel expression in the central nervous system are well established, the expression of individual channel isoforms and their subunit composition in specific types of neurons are still incompletely understood. Therefore a comprehensive characterization of the expression of voltage-gated calcium channels in specific brain regions and well defined neuronal culture systems, in our case low-density cultures of mouse hippocampal neurons, is important. In order to generate a reliable and reproducible expression profile by Taqman quantitative RT-PCR we combine absolute quantitation based on standard curves with relative quantitation based on a variety of endogenous reference genes. To this end we have established PCR-fragment-based standard curves for all high-voltage activated calcium channels and all known auxiliary beta and alpha-2-delta subunits. In addition we are currently validating a series of genes for their suitability as endogenous reference. Quantitative data will be presented that demonstrate (1) which calcium channels and auxiliary subunits coexist in the hippocampus and in cultured hippocampal neurons and (2) what their relative contribution to the calcium channel transcriptome in these systems is. In combination with information on their subcellular distribution, these findings will contribute to our knowledge of the exact molecular composition of the heteromeric channel complexes responsible for distinct neuronal functions. Moreover, expression profiling will serve as baseline for monitoring changes during development and experience-induced modulation of neuronal function.

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Doctoral Program

Molecular Oncology

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28. The GR^{dim}-mutation as a tool for dissecting the pleiotropic actions of glucocorticoids

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Glucocorticoids (GC) induce apoptosis and cell cycle-arrest in acute lymphoblastic leukemia (ALL), however which GC-receptor (GR) target genes are responsible and whether this occurs via transactivation and/or transrepression is unclear. To address this question, we have retrovirally transduced a GR-deficient human T-ALL cell line with wildtype GR and a transactivation-deficient but transrepression-intact GR mutant (GR^{dim}). FACS-analyses showed that GC-sensitivity could be restored by GR^{wt} and by high levels of GR^{dim}. Detailed time course and concentration analyses revealed that the mutant induced apoptosis consistently in an unusual pattern, i.e., at the 48h time point low concentrations of dexamethasone induced high degree of apoptosis and *vice versa* ($5 \times 10^{-9} \text{M} \rightarrow 65\% \pm 7$; $10^{-6} \text{M} \rightarrow 34\% \pm 5$). This suggested that GR^{dim} differentially regulates survival and apoptosis genes in a time and concentration-dependent manner. Moreover, the mutant induced G1 cell cycle arrest earlier and more pronounced than GR^{wt}. These remarkable phenotypes make the GR^{dim} and GR^{wt} transduced cell lines a promising discriminatory model for the identification of genes involved in apoptosis/survival decisions and cell cycle arrest. To exploit this potential, we subjected the model to Affymetrix-based comparative whole genome expression profiling (U133 plus 2.0) and are currently analyzing the resulting microarray data using a number of bioinformatic approaches.

29. Znf307 – Identification of a novel glucocorticoid receptor – interacting protein

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Background: The main goal of this project is to identify and further analyse novel protein interaction candidates of the glucocorticoid receptor (GR), a key transcription factor in the regulation of immune and inflammatory as well as metabolic reactions.

Methods: Therefore, we take advantage of a specialized yeast two hybrid system suitable for transcriptional regulators, the so called “reverse ras recruitment system”, using the entire human GR as bait. Potential candidates are then validated in mammalian cells by coimmunoprecipitation and colocalization experiments followed by functional assays depending on the nature of the prey.

Results: One of the verified interactants is Znf307, a KRAB-box containing zinc finger protein that is colocalizing with the receptor in a speckled pattern in the nucleus. To investigate the site of interaction with the GR, various domains have been subcloned and analysed by fluorescence microscopy and coimmunoprecipitation experiments with antibodies produced during the study. For a detailed analysis of Znf307 mode of action, a cell line, stably expressing inducible shRNA, is being established.

Conclusion: Among the isolated preys are proteins for which interaction with the GR is known, encouraging us to expect that understanding Znf307 will contribute to our understanding of glucocorticoid effects.

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30. Identification and analysis of human cell cycle regulators

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Background: Faithful cellular reproduction relies on exact DNA replication and segregation during mitosis. Although posttranslational modification by CDK-dependent phosphorylation is a key regulatory process in mitosis, many human cell cycle regulators are also controlled on a transcriptional level. Previous studies (Whitfield et al. 2002) have revealed more than 800 cell cycle dependent transcripts in human cells.

Methods: To identify novel regulators of mitosis we have targeted 30 genes, with expression peaks in late G2-phase, by constructing 2-3 conditional shRNA expression vectors/gene and reverse transfection of HeLa cells. For further analysis, cDNAs of target genes were cloned, specific antibodies raised for immunofluorescence and RNAi phenotypes evaluated in detail.

Results: Reverse transfection of HeLa cells using this small shRNA library revealed 2 genes that caused a mitotic phenotype and one, CdcA2, chosen for further analysis. Quantitative real-time PCR and immunoblotting revealed that CdcA2 oscillates during the cell cycle and peaks in mitosis where it becomes strongly phosphorylated. CdcA2 associates with mitotic chromosomes and RNAi caused chromosome segregation defects as well as apoptosis in interphase cells.

Conclusion: By scrutinizing cell cycle regulated genes for mitotic phenotypes by RNAi we have identified CdcA2 as a potential regulator of mitosis and apoptosis.

31. Possible role of GC-target genes in apoptosis and cell-cycle regulation

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Glucocorticoids (GC) have pronounced effects on metabolism, differentiation, proliferation and cell survival. In certain lymphocytes and lymphocyte-related malignancies, GC inhibit proliferation and induce apoptosis. This has led to their extensive use in the therapy of malignant lymphoproliferative disorders. Our lab has addressed the molecular basis of the anti-leukemic GC effects by a comparative gene expression profiling strategy using 13 ALL-children and other biological systems of GC-sensitivity and resistance. This provided a list of 22 genes, representing the most probable GC-regulated candidates. Two particularly interesting members of this list are SNF1LK and PFKFB2. The former has been implicated in cell cycle regulation, the latter is a regulator of glucose metabolism and might play a role in inducing cell death via a non-classical apoptotic pathway.

To test their possible involvement in GC-induced cell death and/or cell-cycle arrest, we exploited the Gateway technology to generate lentiviral constructs for conditional expression of SNF1LK and the 2 major splice variants of PFKFB2. Using these constructs, we established several stable transfected subclones of the human acute lymphoblastic leukaemia cell line CCRF-CEM with tetracycline-regulated expression of SNF1LK and PFKFB2 variants. These clones are currently being tested by FACS analysis for survival and cell cycle progression in the presence and absence of GC.

32. Functional Analysis of hSLA, a Candidate Gene in Glucocorticoid-Induced Apoptosis

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Glucocorticoids (GC) like dexamethasone are widely used as core element of chemotherapy for lymphoid malignancies because they have potent proapoptotic properties but the exact mechanism by which GC cause apoptosis and affect cell cycle is still elusive. In our recent Affymetrix based whole genome comparative expression profiling, we had defined highly potent candidate genes for apoptosis. Human SLA (src like adaptor) was one of the most promising candidate as it was regulated in at least 10 out of 13 ALL children as well as in an adult ALL patient and two GC sensitive ALL cell lines. To investigate the role of hSLA in glucocorticoid-induced apoptosis and cell cycle arrest we employed Gateway Cloning Technology and over expression/knock-down systems recently developed in our lab. hSLA coding sequence was PCR amplified using attB sites and then inserted into a compatible construct, pDONR207, by BP recombination. The resultant pENTR207-SLA was subsequently used to generate an HIV derived lentiviral vector, pHRtet-CMV-SLA-ires-GFP by LR recombination. This construct was then utilized to transduce the widely used leukemic T-ALL model CCRF-CEM-C7H2 and B-ALL model preB697 with the hSLA gene. To produce viral particles for transduction, 293 T were used as packaging cell line. Supernatants were subsequently used to infect CEM-C7H2 2C8 cells developed by our lab for conditional expression. The initial investigations revealed that hSLA changed the morphology of packaging cells but the role of this gene in CCRF-CEM cells still needs to be defined.

33. Expression and Localisation of Proteins of the IGF axis in the Prostate and Prostate Cancer

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Insulin like growth factors (IGFs) and their binding proteins (IGFBPs) play an important role in prostate cancer. Production and localisation of IGF-factors within the prostate is poorly described so far.

We analysed the expression of IGF-factors in a panel of cells and cell lines deriving from the prostate and prostate cancer by Real Time PCR. Additionally we localised IGFBP 3 and 5 in tissue sections of benign and malignant prostate samples. IGFBP-3 secreted by the prostate was determined measuring a set of ejaculate samples of patients after vasectomy by sandwich ELISA.

We could show that in the prostate the stromal cells are the main producers of IGF-axis factors, especially of IGF II and the IGFBPs 3 and 5. The distribution of these binding proteins within the prostate suggests that they accumulate in benign and malignant epithelial cells. Additionally IGFBP-3 can be found in a concentration up to 2 µg/ml in the ejaculate of patients after vasectomy. Our results support the importance of the stimulation of epithelial cells via stromal cells through the IGF-axis. The production of IGF I seems to be low in prostatic cells but can be enhanced in some progressed cancer cells resulting in additional autocrine stimulation.

Doctoral Program

Musculoskeletal Science

34. Prediction of Countershock Success Employing Single Feature Analysis from Ventricular Fibrillation Frequency Bands or Feature Combination Using neural Networks

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Background: Targeted defibrillation therapy is needed to optimize survival chances of ventricular fibrillation (VF) patients. This study was performed to develop improved defibrillation prediction features in VF electrocardiogram (ECG) data from cardiac arrest patients.

Methods: From 197 patients with in-hospital and out-of-hospital cardiac arrest, 770 ECG recordings of countershock attempts were analyzed. Ten preshock VF-ECG features in the time and frequency domain were calculated from a 2.5 sec VF-ECG segment prior to shock and were retrospectively tested for outcome prediction. Using band pass filters, the ECG spectrum was split into various frequency bands of 2-26 Hz bandwidth in the range of 0-26 Hz. Neural networks were used for single feature combinations to optimize counter-shock success prediction. Areas under curves (AUC) of receiver operating characteristics (ROC) were used to estimate prediction power of single and combined features.

Results. The highest ROC-AUC of 0.863 was reached by median slope in the interval 10-22 Hz resulting in a sensitivity of 95% and a specificity of 50%. Neural networks combining single predictive features were unable to increase outcome prediction.

Conclusion. Features combination using neural networks did not further improve outcome prediction of well performing single features resulting from frequency band segmentation of human VF-ECG.

Doctoral Program

Neuroscience

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35. Does unilateral temporal lobe epilepsy affect cognitive estimation?

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Background: Cognitive estimation is a common activity in everyday life, and it is defined as the capacity to give an adequate answer to a problem when the exact solution is not readily available. We examined cognitive estimation in patients with temporal lobe epilepsy (TLE) with a multi-dimensional estimation task. We also assessed whether possible deficits are more pronounced in left or right TLE patients.

Method:

Participants:

31 patients with unilateral TLE:

a) LTLE (N= 13; Mean age= 33.62, Mean years of schooling= 11.39; Mean Verbal IQ= 98.92)

b) RTLE (N= 14; Mean age= 37.14, Mean years of schooling= 10.64; Mean Verbal IQ= 100.5)

In addition to a comprehensive neuropsychological examination participants took the Test of Cognitive Estimation, which examines performance on different dimensions of cognitive estimation, namely, size, weight, quantity, time.

Results: RTLE patients had significantly lower scores than LTLE on the size estimation task of the TKS.

Conclusion: Although patient groups did not differ in their overall scores, a closer analysis shows specific difficulties.

RTLE patients could have a deficit on estimation tasks that tap retrieval of visual information from semantic memory. A follow up of these results may help us elucidate the association between the lateralization of temporal lobe dysfunction and cognitive estimation.

36. Signaling mechanisms controlling sympathetic cholinergic differentiation

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We study cellular signaling mechanisms controlling the acquisition of neurotransmitter phenotypes using sympathetic neurons as our model. This cell type can undergo a switch from noradrenergic to cholinergic neurotransmission during development. This plastic phenomenon can be triggered *in vitro* by stimulation with growth factors, such as ciliary neurotrophic factor (CNTF), which evokes an increase in cholinergic marker genes expression and a concurrent decrease in noradrenergic markers. By contrast nerve growth factor (NGF) promotes the noradrenergic phenotype in these cultures.

Primary neurons from rat and chicken sympathetic ganglia were cultured in defined medium supplemented with NGF, in the presence or absence of CNTF. The activation of p38 and Erk1/2 MAP kinases was investigated using antibodies which selectively recognize activated isoforms of these proteins in Western Blot. Specific inhibitors of the p38 (SB202190) and Erk1/2 (UO126) signaling pathways were added to the cultures prior to CNTF treatment. Their effect on marker gene expression levels was monitored by real-time RT-PCR. These experiments identified the p38 MAP kinase signaltransduction cascade to be involved in the cholinergic differentiation of sympathetic neurons triggered by CNTF. Further they indicate a role of the Erk1/2 cascade in regulating their noradrenergic properties in response to NGF.

37. Characterization of Cell Populations in Cerebrospinal Fluid of Patients with Multiple Sclerosis

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Background: Recent studies detected B-cells in the cerebrospinal fluid (CSF) of patients with MS and other neurological inflammatory diseases (IND), but not in non-inflammatory neurological diseases (OND). Moreover, raised intrathecal immunoglobulin synthesis and oligoclonal IgG bands (OCB) are observed in >90% of all MS patients. The aim of this study was to analyze CSF cell populations in patients with clinically isolated demyelinating syndromes (CIS; n=15), relapsing-remitting (RRMS; n=11) and primary progressive (PPMS; n=6) MS compared to IND (n=13) and OND (n=17) in order to determine differences in the distribution of B-cell subsets and to address their role in neuroinflammation.

Methods: CSF was obtained by standard diagnostic lumbar puncture. CSF cells were immediately stained with fluorochrome-labeled antibodies to human leukocyte surface antigens and were analyzed via three-color flow cytometry.

Results: We found a significantly increased number of CSF CD19+ B-cells in CNS inflammation (CIS, RRMS, PPMS and IND) and CD19+CD138+ plasma blasts in MS. CSF B-cells were higher in CIS and RRMS than in PPMS. Furthermore, CSF B-cells were associated with OCB and increased intrathecal IgM/IgG production.

Conclusion: Our results reveal an accumulation of B-cells (CD19+) and plasma blasts (CD19+CD138+) in the CSF of relapsing-remitting MS patients thus providing further evidence for their complex role in the neuroinflammatory pathogenesis of MS.

38. Neural correlates of routine activities and navigation in well-known surroundings: the involvement of the hippocampal complex

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Background: Everyday action and mobility is of general practical and social importance. The present study aimed to investigate brain activation elicited by everyday action and to compare it with a navigation task, especially with regard to the involvement of the hippocampal complex. While the hippocampal complex plays a crucial role in navigation it is unclear which role it might play in the imagination of everyday activities.

Methods: Twenty-one volunteers participated in an fMRI experiment which consisted of two scanning runs, one for each imagination task. The navigation task required participants to mentally navigate through personally well-known surroundings. The everyday activities task required the imagination of given everyday routine activities. Both tasks were performed for 28 s alternating with a baseline condition.

Results: Compared to the baseline condition, both experimental conditions activated the hippocampal complex. Importantly, both tasks strongly activated the hippocampus with a dominance of the left hemisphere. The comparison between both tasks shows that navigation activated the hippocampal complex more strongly than imagination of everyday activities.

Conclusion: The results suggest that the hippocampal complex is not only crucial for navigation but also for the retrieval of often practiced activities. The parahippocampus seems to be more specifically involved in navigation.

39. Fibroblast growth factor receptor 1 mediated neurite outgrowth is regulated by Sprouty

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Background: Basic fibroblast growth factor (FGF-2) is induced in response to peripheral nerve lesion. In adult sensory neuron culture, FGF-2 isoforms efficiently promote axonal elongation. FGF-2 activates fibroblast growth factor receptor 1 (FGFR1) and induces axonal growth through Ras/MAP kinase activation. The negative feedback regulator Sprouty attenuates FGFR signalling by inhibition of MAP kinase activation. Sprouty isoforms represent suitable targets for interference in order to maintain FGFR signalling for improved axonal regeneration.

Methods: PC12 cells and neurons were transfected with FGFR1 isoforms and with Sprouty shRNAs using the AMAXA electroporation system. Neurite outgrowth was measured after growth factor addition. Sprouty expression and shRNA induced Sprouty down-regulation was analyzed by quantitative real time PCR.

Results: We examined the effects of FGFR1 overexpression and Sprouty down-regulation on FGF-2 dependent neurite outgrowth. Pre-treatment with NGF enhanced the sensitivity for FGF-2 induced differentiation by up-regulation of FGFR1. FGFR1 overexpression promoted FGF-2 induced neurite outgrowth in PC12 cells and neurons. Expression of endogenous Sprouty mRNA was regulated by FGF-2 and NGF. Down-regulation of Sprouty by shRNA treatment enhanced neurite outgrowth in PC12 cells.

Conclusion: Taken together, these results indicate that NGF, FGFR1 overexpression and Sprouty down-regulation promote FGF-2 induced neurite outgrowth.

40. NogoA/RTN-4A is Phosphorylated by Cyclin-Dependent Kinase 5

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NogoA/RTN-4A, a member of the Reticulon protein family, is predominantly expressed in the mammalian central nervous system. Originally cloned as a myelinderived neurite growth inhibitor, Nogo-A/RTN-4A has been studied mostly in the context of axonal regeneration and structural plasticity in the adult CNS. In the embryonic brain, however, NogoA is thought to perform intracellular functions that are unrelated to its inhibitor character. Here we show that in Western Blot analysis of brain lysates of different developmental stages after treatment with Calf Intestinal Phosphatase, phosphorylation of NogoA/RTN-4A occurs from embryonic day 12 through postnatal day 8. This time window coincides with neuronal migration during brain development. Moreover, we could identify Nogo-A/RTN-4A as a direct substrate for p35/cdk5, a member of the cyclin-dependent protein kinase family, which has been functionally linked to neuronal migration and neurite outgrowth via its action on actin cytoskeleton. Mass-spectrometry and Western blot analysis indicate several phosphorylation sites in vitro and in intact cells.

These data indicate that phosphorylation of Nogo-A/RTN-4A by members of the cyclin-dependent kinase family is a conserved property of Nogo-A/RTN-4A and suggest that p35/cdk-5-dependent phosphorylation of NogoA may play an important physiological role in neuronal migration.

41. Loss of dopaminergic responsivity in the double lesion SND/MSA-P rat model

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Background: The parkinsonian variant of Multiple System Atrophy (MSA-P) is characterized by a loss of dopaminergic neurons comparable to that found in Parkinson's disease. In addition, progressive loss of striatopallidal projections is thought to account for L-Dopa unresponsiveness in MSA-P. Whereas histological features of MSA-P have been successfully reproduced in the double lesion SND/MSA-P rat model, loss of L-dopa responsivity has so far not been demonstrated in this model.

Methods: 15 male Wistar rats received a stereotaxic unilateral 6-hydroxydopamine (6-OHDA) injection into the left medial forebrain bundle, followed by two stereotaxic injections of 75 nmol quinolinic acid (QA) each into the ipsilateral striatum. Forelimb akinesia was assessed by the stepping and the cylinder test without drugs and following dopaminergic stimulation with L-Dopa (8mg/kg i.p.). For lesion assessment, sections were stained with TH and DARPP-32.

Results: The marked contralateral forelimb stepping deficit observed following 6-OHDA improved significantly after challenge with L-Dopa ($p < 0.001$). This responsivity to L-Dopa was abolished by the subsequent striatal QA lesion. In the cylinder test, the marked asymmetry observed after unilateral 6-OHDA lesioning was reversed by L-Dopa to baseline levels. Following QA lesion animals almost exclusively used the ipsilateral paw, with cylinder test performance under L-Dopa failing to reach baseline ($p = 0.001$) or 6-OHDA + L-Dopa ($p = 0.002$) levels. Nigral cell loss ($90\% \pm 5\%$) correlated with both stepping deficit ($r = 0.561$, $p = 0.015$) and responsivity to L-Dopa ($r = 0.608$, $p = 0.008$) as well as cylinder test results ($r = 0.656$, $p = 0.005$). Lesion size of the dorsal striatum correlated significantly with the loss of L-Dopa response ($r = 0.593$, $p = 0.01$) in the stepping test.

Conclusions: These findings contribute further to the behavioural characterisation of the double lesion rat model of MSA, improving its value in the evaluation of future neurorestorative strategies.

42. Immunohistochemical characterization of the rat subicular-entorhinal complex in the kainic acid animal model for human temporal lobe epilepsy (TLE):

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The subiculum is the main output region of the hippocampus. It receives its main inputs from hippocampal region CA1 and sends major projections to the deep layers of the entorhinal cortex (EC). In contrast to the Ammon's horn, the subiculum remains almost entirely preserved in human TLE.

The aim of this study was to characterize changes in neurons of the subicular-entorhinal complex and its projections after kainic acid (KA) induced seizures. Neurodegeneration was assessed by Nissl staining and NeuN-immunoreactivity (IR), antibodies for GFAP and OX-42 were used to visualize glial proliferation. We used the neuropeptides NPY and cholecystokinin-8 (CCK-8) as markers for different neuronal subclasses.

We observed neurodegeneration and glial proliferation in all subregions of the subicular-entorhinal complex, especially in the proximal half of the subiculum and in layers III and V/VI of the medial entorhinal cortex. After KA-induced seizures, NPY-immunoreactive terminals were especially dense in the lateral entorhinal cortex, in the parasubiculum, and in the subiculum. Several NPY-ir pyramidal cells were located in the subiculum and in hippocampal CA1.

In contrast to human TLE, the subiculum is severely damaged after KA-induced seizures. NPY is expressed in principal cells throughout the whole formation.

43. Deletion of Y2-Receptors in Y2^{lox/lox} Mice by Local Infusion of Adeno-Associated-Virus (AAV) Vectors Expressing Cre-Recombinase and Subsequent Behavioural Testing.

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Neuropeptide Y (NPY) is abundant in the central and peripheral nervous systems. It acts through Y1, Y2, Y4 and Y5 receptors and is involved in a variety of brain functions, including regulation of appetite and anxiety. When applied locally into the amygdala, NPY exerts an anxiolytic action, presumably mediated by Y1 receptors. In contrast, stimulation of Y2 receptors causes anxiety. Depletion of Y2 receptors induces an anxiolytic phenotype, possibly by abolishing the release-inhibiting action of presynaptic Y2 receptors.

We now established site-specific deletions of Y2 receptors in conditional Y2^{lox/lox} mice by local injection of an AAV2/2-Cre-recombinase vector into the hippocampus, septum or amygdala. As controls, an AAV2/2-GFP vector was injected in Y2^{lox/lox} mice at the same sites. Expression of Cre-recombinase and GFP was demonstrated by *in situ* hybridization and immunohistochemistry. Deletion of Y2 receptors and Y2 mRNA was visualized by receptor autoradiography and *in situ* hybridization, respectively. It was neuron-specific and restricted to the injection sites. After bilateral injection of AAV2/2-Cre vector into the amygdala, mice showed a tendency towards an anxiolytic phenotype in the light-dark test for anxiety. A similar change in phenotype was also observed after intra-amygdaloid injections of an AAV2/1-Cre vector. In these mice an even higher rostro-caudal diffusion and a higher number of the Cre-recombinase mRNA expressing neurons was seen. No anxiolytic effect was detected in mice after intra-hippocampal or intra-septal injections of AAV vectors. The experiments indicate that the anxiolytic effect induced by Y2 receptor deletion may be generated in the amygdala.

44. Expression Levels and Localization in Lipid Rafts of Nogo-A, NgR1, p75^{NTR} and RhoA in the Cerebellum of Wild Type and Knockout Animals.

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Background: Many receptor complexes are formed as signalling platforms in microdomains of the cell membrane, known as lipid rafts. Lipid rafts remain insoluble upon detergent extraction and float in a sucrose gradient after ultracentrifugation. We investigate the signalling mechanisms of myelin-derived nerve growth inhibitors. We specifically analyze the distribution in detergent resistant membranes (DRM) and the total expression levels of Nogo-A, NgR1, p75^{NTR} and RhoA in the developing mouse cerebellum and cultured mouse cerebellar granule neurons (CGN) from wild type and knockout (*ngr1* *-/-*, *ngr2* *-/-*, *p75^{NTR}* *-/-*) animals.

Methods: Cerebella of different developmental stages and CGN in culture for different time periods were subjected to detergent extraction using TritonX-100. Upon fractionation into detergent soluble and insoluble proteins via sucrose gradient ultracentrifugation analysis was completed with SDS-PAGE and Western-Blotting.

Results: NgR1 gets strongly upregulated over time in CGN in contrast to cerebellum development. A presumably less glycosylated form of Nogo-A is specifically reduced in *ngr1* *-/-* and *ngr2* *-/-* animals at later developmental stages.

Conclusion: Other than expected, no compensatory effects on the expression of the investigated proteins and their localization in DRM have been observed in either knockout strain. We will further investigate the differential modification of Nogo-A in vivo.

45. Structural and functional characterization of the neurite growth inhibitor Myelin-associated glycoprotein (MAG)

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Regeneration of injured nerve fibres in the adult mammalian central nervous system (CNS) is partly restricted by myelin-derived inhibitors like MAG, Nogo or OMgp. MAG is a transmembrane member of the immunoglobulin-superfamily with five Ig-domains. The current model of MAG-mediated signalling suggests that MAG binding to its receptors NgR1 or NgR2 induces the formation of a ternary receptor complex containing the neurotrophin receptor p75^{NTR} and LINGO-1. Although the precise mechanisms are presently unknown, the inhibitory effect of MAG on neurons is mediated by RhoA, an intracellular regulator of the cytoskeleton. RhoA activation correlates with the inability of axons to elongate.

The goal of our present study was to identify the inhibitory domain of MAG and to characterize its interaction with NgR1 and/or NgR2. For this purpose various deletion or swapping mutants of the MAG molecule were generated and analysed for their binding and neurite growth inhibitory properties on neurons of wildtype and NgR1^{-/-} or NgR2^{-/-} mice.

Our data demonstrate that the neurite outgrowth inhibitory domain of MAG resides in the Ig4 and Ig5-domains of the extracellular part of MAG and that both domains are required to render neurons fully sensitive to MAG, depending on the receptor composition of various neuronal subtypes.

46. Immunomodulation of Macrophage and Dendritic Cell Function by Myelin – Role of CD14

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Background: Widespread inflammatory infiltrates and multifocal demyelinating lesions within the central nervous system are the pathological hallmarks of multiple sclerosis (MS). Monocytes and macrophages constitute the major subset of infiltrating cells within MS lesions and play an important role in the inflammatory process, but they also mediate the non-inflammatory removal of apoptotic cells and myelin debris. The aim of this study is to investigate whether human myelin is able to modulate the function of macrophages and dendritic cells (DC) through CD14, a receptor of the innate immune system.

Methods: Human monocytes, macrophages and DC were activated with lipopolysaccharide (LPS) and/or human myelin. Cell activation was measured by CD14 and HLA-DR expression using FACS. Furthermore, the production of IL-6 and IL-10 was analyzed by ELISA.

Results: We found a CD14 mediated, time- and dose-dependent increase in the expression of CD14 and HLA-DR as well as an enhanced secretion of IL-6 and IL-10 after stimulation with myelin.

Conclusion: Our results indicate an important role of CD14 and the innate immune system in the activation of monocytes by myelin. The effects on macrophages and DC are currently analyzed.