7th Meeting of PhD students

Program

12.15 – 14.15	Poster Session I
14.15 – 15.00	Coffe Break
15.00 – 16.30	Poster Session II
16.30 – 17.00	Coffe Break
17.00 – 18.00	Key Lecture: Prof. Jürgen KNOBLICH
18.00:	Comedian "Der Koschuh" and Award Ceremony / Special Prize
18.30:	Buffet and Drinks
20.00:	AFTER CONGRESS PARTY at UNI-Café, Innrain 55

Special thanks to our sponsors who donate our POSTER AWARDS and make this event possible!

Dear Colleagues,

it is a great pleasure to welcome you all to the 7th meeting of PhD-Students of the Medical University of Innsbruck. After the positive feedback on doing a joint meeting with PhD-students of the Life Science Disciplines of our partner university, I am very proud that this is maintained to strengthen the interdisciplinary approaches of all PhD-programs of the Medical University as well as PhD-programs of the Leopold-Franzens-University.

The work of PhD-students is essentially for scientific progress at universities and with this meeting PhD-Students get the chance to presenting achievements to their current projects and get routined talking in front of a knowledgeable audience.

Discussions arising with other students and also with fellow scientists about the presented data will lead to inspiration, new ideas and therefore will help to assess the quality of research. Furthermore, meetings, as the PhD-meeting itself, demonstrate the scientific community's appreciation of the work of PhD-students.

I am very happy that PhD-students are a fundamental part of this scientific community and wish you a successful meeting. Seeing the results of your research published in this abstract book makes me proud of your achievements.

Finally, I want to thank all who have been involved in organizing this meeting and wish you all the best for your future projects and careers.

Sincerely

Univ.-Prof. Dr. Herbert Lochs
Rector

Dear Colleagues!

Hardly any research field encompasses as many different disciplines as the so called Life Sciences. Therefore it is obvious that knowledge exchange and networking is particulary important for all researchers dedicated to this branch of research. Due to this I am really happy that the formal border between the University of Innsbruck and the Medical University, which became an autonomous institution in 2004, did not affect the collaboration of our scientists: The 7th meeting of PhD students from the two institutions is a very good example for that.

This meeting, where PhD students of the University of Innsbruck and the Medical University of Innsbruck meet to discuss current research projects, follows a long history of successful interdisciplinary cooperations. As Rector of the University of Innsbruck I greatly appreciate these efforts and I wish all participants a successful meeting with fruitful discussions.

Yours sincerely,
Univ.Prof. Dr. Dr.h.c. mult. Tilmann Märk
Rector of the University of Innsbruck

Dear doctoral students, dear colleagues:

In the nine years of its existence the Doctoral program at the Medical University Innsbruck (MUI) has grown to an enrolment of over 280 students. Thus, PhD students represent about 10% of the total students of the MUI, matching in numbers the studies of dental medicine. This high number of PhD students is particularly remarkable, as it reflects an equally high number of doctoral positions, which are for the most part funded by peer-reviewed research grants like FWF and EU projects. Indeed much of the scientific output of the MUI is the direct result of research carried out by our PhD students. Clearly, our doctoral research program has become a vital factor for the high international reputation of the MUI as a European biomedical research center. Moreover, it indicates the high commitment of the MUI to top research training in biomedical sciences as an import aspect of state-of-the-art medical education, which ultimately creates the bases for the advancement of clinical practice.

This also is the message successfully conveyed by the *Meeting of the Doctoral Students* organized annually by the student representatives of the Medical University. This science fair with poster presentations by our PhD students and a guest lecture by a prominent scientist has already become a valuable tradition in Innsbruck. It provides our students with an opportunity to present their most recent results to a highly qualified audience of scientist, and thus to practice their presentation skills and perhaps make valuable contacts for future career steps. It allows students and PIs to directly compare their scientific achievements with those of peers, and to compete for one of the attractive poster prices. Importantly, by presenting our students' scientific achievements to a broad audience within and outside the University, the *Meeting of the Doctoral Students* gives a strong sign of life of the dynamic research community at the MUI and in the life science disciplines of the LFU.

However, this annual event is only the highly visible “peak of the iceberg” of our PhD student representative's work. Throughout the year they are available for the concerns of their peers, they run an informative homepage, actively participate in the regular meetings of the PhD coordinators and they communicate the PhD student's concerns to the vice rector and the leadership of the MUI.

Therefore, I want to take this opportunity to thank our PhD student representatives for their initiative and hard work throughout the year as well as for organizing this science fare. Furthermore, I encourage all colleagues and faculty members to participate at this year's *Meeting of the Doctoral Students*, get to know our doctoral students and share their excitement about science.

Ao.Univ.-Prof. Dr. Bernhard E. Flucher
Coordinator of the PhD programs at the Medical University Innsbruck

Dear member of the faculty and colleagues!

More than 7 years ago Innsbrucks two universities have been separated officially. But this does not apply to scientific collaborations, joint projects, and friendships. With the 6th PhD Congress we want to go one step further to strengthen the bond between the doctoral students of the Life Science Disciplines of the University of Innsbruck and Innsbruck Medical University. We want to foster students contact and gain synergies. This year we are very proud to have a new record of 102 submitted abstracts and the meeting is already established as an anchor of the Innsbruck scientific community. We are further looking forwad to the opening of the new Center for Chemistry and Biomedicine (CCB) which is an important step for both universities.
By this means we want to thank all participating students and guests, all the jury members, who spent their precious time to judge the work of the students and all sponsors and coworkers who make this event possible!
Finally, one should keep in mind that doing a PhD is not just researching in a lab; it is also exchanging ideas and meeting people from different countries and cultures. Therefore, it is clearly not just an intellectual, but also a socio-cultural challenge. The annual PhD Congress is strongly supporting this noble idea.

Daniela Trimmel, Christiane Maria Bauer, Dominik Pesta, Jennifer Gebetsberger, Agnieszka Martowicz, Martin Bodner, Peter Knetsch, Gurjot Kaur, Martha Nowosielski, Barbara Meissner, Kerstin Schmidt and Alexander Keiler (picture from left to right)

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1 Loss of Nogo Receptor Homolog NgR2 Causes Defects in Peripheral Sensory Innervation and Mechanical Pain Sensation

B. E. Bäumer[1], A. Rjabokon[2], M. Kress[2] and C. E. Bandtlow[1]

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The Nogo-66 receptor homolog NgR2 is a member of the Nogo-receptor family and is suggested to impair axonal regeneration of injured nerve fibers and to restrict structural plasticity in the uninjured brain. In the PNS, NgR2 is expressed in dorsal root ganglia (DRG) neurons, but its physiological relevance remains unknown. In this study we examined how absence of NgR2 affects anatomy, gene expression and nociceptive behavior of cutaneous DRG sensory afferents in NgR2 deficient mice.
Interestingly, NgR2-/- mice were markedly hypersensitive to noxious mechanical but not to heat stimuli. Compared to wt mice, no change in the percentage of nociceptive (IB4-binding and CGRP-positive) and non-nociceptive (NF200-positive) DRG neurons was found in NgR2-/- mice, consistent with unaltered soma size of sensory neurons. Strikingly, the number of nonpeptidergic (IB4-binding) free nerve endings in the footpad epidermis was about 40% higher in NgR2-/- mice than in wild-type controls. In contrast, the density of CGRP-positive epidermal sensory fibres remained unaffected. We could show that the cutaneous hyperinnervation was not associated with changed expression levels of neurotrophins in the skin or their cognate receptors in DRG neurons.
However, specifically IB4 binding dissociated NgR2-/- DRG neurons exhibit a higher branching ability than wt neurons *in vitro*. Additionally NgR2-/- neurons were significantly less inhibited by chondroitin-sulfate proteoglycans (CSPGs) such as versican, which is enriched at the dermal epidermal border but not by other inhibitory substrates like Ephrin-A4. On alternating stripe choice assays we were able to show that even the number of entries into the inhibitory stripes was remarkably increased for NgR2-/- neurons.
Finally these data demonstrate that NgR2 is required for proper cutaneous sensory innervation of the skin and suggest that it limits axonal outgrowth and branching mediated through CSPGs specifically for IB4-binding neurons.

2 Endocytic Trafficking of Fibroblast Growth Factor Receptor 1 in the Human Glioma Cell Line U373

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Background: Fibroblast growth factor receptors (FGFRs) are among the most important receptor tyrosine kinases responsible for tumor growth. As transmembrane molecules they trigger intracellular signaling pathways upon extracellular binding of their ligands FGF, leading to stimulation of the Ras/Raf/Erk pathway and subsequent proliferation. Down-regulation of these receptors and concomittant inhibition of their signaling pathways represents one of the targets of new treatment strategies for multiple tumors, including glioblastoma.
Methods: We are investigating the degradation pathway of FGFR1 in human glioma cells (U373), which are transfected with fluorescent FGFR1 constructs (eGFP- or mCherry-tagged), and colocalization of the receptor is determined with different vesicular markers (for early/late endosomes and recycling endosomes as well as lysosomes). Fluorescence microscopy is performed with a confocal microscope (Leica SP5) or with structured illumination technique (Zeiss AxioObserver ApoTome), and subsequent deconvolution of 3D image stacks is performed with Huygens Deconvolution Software. Additionally, alteration of the FGFR1 trafficking pathway by the lysosomal inhibitor leupeptin is studied.
Results: Our findings suggest that FGF-2 enhances colocalization of FGFR1 with early, late and recycling endosomes as well as with lysosomes. The lysosomal inhibitor leupeptin leads to receptor accumulation in late endosomes and lysosomes. Analysis of vesicle distribution shows an accumulation of recycling endosomes in the perinuclear region, while late endosomes and FGFR1-containing vesicles are homogeneously distributed throughout the cell.
Conclusion: Visualization and analysis of cellular FGFR1 distribution under different treatment conditions was performed. This leads to a better understanding of receptor trafficking and could eventually help to develop new treatment strategies to reduce RTK-mediated tumor growth.

3 Axon elongation is enhanced in sympathetic neurons obtained from heterozygous Sprouty2 knockout mice

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Background: Sprouty2 (Spry2) is known as negative feedback inhibitor of receptor tyrosine kinase signaling pathways. Four isoforms (Spry1-4) have been identified in mammals. Spry2 is the major isoform expressed in peripheral neurons. Down-regulation of Spry2 in adult sensory neurons obtained from the rat dorsal root ganglion (DRG) promotes axon growth i*n vitro* (Hausott et al., 2009). Recent studies from our group provided evidence that neurite outgrowth of sensory neurons obtained from Spry2$^{+/-}$ or Spry2$^{-/-}$ mice is significantly enhanced. Interestingly, Spry2$^{+/-}$ neurons exhibited enhanced axon elongation, whereas Spry2$^{-/-}$ neurons showed a tendency towards axonal branching when compared to wildtype (wt) DRG neurons.

Methods: Here, we investigated axon growth in primary neuron cultures obtained from superior cervical ganglia (SCG) as an in vitro model of visceromotor neurite outgrowth.

Dissociated SCG neurons from Spry2$^{+/-}$ and wt pups (postnatal day 7) were plated on a poly-D-lysin/laminin substrate and treated with 5 ng/ml nerve growth factor for 24 hours. The neurons were fixed by 4% paraformaldehyde and identified by antibodies against neuron-specific class III beta-tubulin (Tuj1). Morphometry software was used to measure the following axon outgrowth parameters: the maximal distance (length of the longest axon), total axon length and the number of axonal branch points.

Results: SCG neurons from Spry2$^{+/-}$ mice revealed a significantly enhanced maximal axon length when compared to wt littermates, whereas no differences were observed with regard to the total axonal length and axonal branching in the presence of NGF.

Conclusion: Taken together, the results demonstrate that Spry2$^{+/-}$ mice reveal a specific axonal elongation phenotype, which may have consequences for the stimulation of long-distance regeneration in clinically relevant nerve lesion models.

4 Neural correlates of number processing in 6-7 year old former preterm children

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The prevalence of learning disabilities, as developmental dyscalculia (DD), within preterm children (without neurological complications) is very high (approximately 40%). Therefore preterm children are among a population at risk to develop DD. The aim of this study was to identify neural correlates of number processing in 6-7 year old former preterm children.
We tested 15 former preterm children regarding their number processing and calculation skills using the TEDI-MATH test battery. The same children participated also in an fMRI paradigm with a numerical comparison task (Which number is higher? => 5 vs. 2).The numerical comparison task is an approved method to determine numerical comprehension. A robust effect regarding reaction time is the numerical distance effect (NDE), which is characterised by a negative correlation between numerical distance and reaction time. The NDE depends on performance and is modulated primarily by (intra)parietal brain regions.
Our results show, that 6-7 year old former preterm children display a robust NDE. The NDE in our experimental group did not correlate with math performance. Interestingly, the strength of the fMRI-signal on the other hand seemed to be related to number processing skills. Lower performance in TEDI-MATH was associated with increased brain activity in number relevant (fronto-parietal) brain regions during the fMRI paradigm. The reported negative correlation between performance and brain activity supports the assumption of compensatory neuronal mechanisms.
To our knowledge our study is the first to examine the number processing skills of former preterm children with an fMRI paradigm. The increased brain activity of mathematical low performers in number relevant brain regions suggest the recruitment of a compensatory fronto-parietal brain network.

5 Investigating the Molecular Basis of a Novel Anxiolytic Affect in Nogo Receptor 2 Knockout Mice.

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Background: Nogo receptor 2 (NgR2) is a member of the Nogo receptor family, members of which are known to bind inhibitors of axonal regeneration in the CNS. Here we investigated the role of NgR2 in emotional behavior.
Methods: A NgR2 knockout mouse line was obtained. A battery of behavioral tests were carried out for anxiety-related behavior, depression-related behavior, and learning and memory. *In situ* hybridization was used to confirm NgR2 expression. RT-qPCR and immunohistochemistry were used to examine changes in gene expression in the brain.
Results: Under mild stress conditions, NgR2-/- mice have a phenotype of reduced anxiety and depression. An anxiolytic effect was seen in the open field test, which was further confirmed by decreased fear expression in contextual conditioned fear. A decrease in depressive-like behavior was observed in the tail suspension test. NgR2 was highly expressed in the hippocampus, cortex, basolateral amygdala and posterior amygdala of wildtype mice, confirming previous reports. A significant increase of activating transcription factor 3 (ATF3), was seen in the dentate gyrus of NgR2-/-. Significant increases in hippocampal mRNA of insulin-like growth factor-2 (IGF-2), Prolactin receptor (Prlr) and the glucose transporter Slc2a12 (GLUT12) were also observed. There were no significant changes in NPY, NPY-Y1, NPY-Y2 or BDNF in the hippocampus or amygdala of NgR2-/-. A reduction in proliferating and immature/migrating cells in NgR2-/- dentate gyrus was also observed, suggesting reduced neurogenesis.
Conclusions: We report reduced anxiolytic and depressive-like behavior in NgR2 knockout mice, alongside increases in hippocampal expression of ATF3 and IGF-2. Reports of these genes in regulating neuroprotection and fear conditioning provide some clues for potential mechanisms, as do results suggesting changes in hippocampal neurogenesis. These mechanisms are under further investigation. NgR2 may represent a novel pathway in the modulation of anxiety or depression related behaviors.

This project is funded by the FWF.

6 Ryanodine Receptor 2 (RyR2) is a Novel Binding Partner of Reticulon1-A

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Background: Reticulons (RTNs) are type II proteins associated with the endomembrane system of eukaryotic cells. While biochemical evidence from non-neuronal cells suggest that RTNs may play a role in regulation of ER membrane curvature [1-3], the physiological function of RTNs in the brain remains elusive. Here we sought to identify binding partners of RTN1-A, the largest isoform of the *rtn1* gene, primarily expressed in principal neurons of the CNS.

Methods&Results: Employing a mass spectrometry-based screen to search for RTN1-A binding partners, we identified cardiac ryanodine receptor 2 (RyR2), the predominant RyR isoform in the brain. *In vitro* glutathione *S*-transferase pull-down and *in vivo* co-immunoprecipitation studies using rat cerebellar lysates confirmed an interaction between RyR2 and RTN1-A; in addition with co-immunoprecipitation of RyR2 with RTN1-A after heterologous expression in HEK 293 cells. Immunocytochemistry revealed that RyR2 co-localizes with RTN1-A in vesicles and ER tubules in primary hippocampal neurons. Moreover, immunohistochemistry indicates a codistribution of RTN1-A and RyR2 in cerebellum and hippocampus. We identified and further characterized the RyR2 interacting domain within RTN1-A. Present experiments are aimed to investigate the impact of RTN1-A on the physiological function of RyR2 in neurons.

Conclusion: Taken together, these findings suggest RTN1-A as a novel interaction partner of RyR2 in brain and raise the intriguing possibility that RTN1-A participates in RyR2-mediated regulation of intracellular calcium homeostasis, through protein–protein interaction.

References

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Acknowledgements

This study is supported by the FWF, within graduate program Signal Processing in Neurons (SPIN).

7 Conditioned Place Preference for Social Interaction: Contribution of Sensory Modalities

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Background: A main challenge in the therapy of drug dependent individuals is to help them reactivate interest in non-drug-associated activities. Social interaction with a gender- and weight-matched conspecific was found to reverse conditioned place preference (CPP) from cocaine to social interaction, and to prevent cocaine-induced reacquisition/reexpression of cocaine CPP in Sprague Dawley rats (Fritz et al 2011, Addiction Biology 16, 273-284). In the present study, we investigated which of the sensory modalities of the composite stimulus 'social interaction' contributes most to the rats' preference for it.

Methods: Early-adult (6 wk) male Sprague Dawley rats were CPP-trained for 15-min episodes of social interaction with a weight- and gender-matched male conspecific. By manipulating the topography of the CPP apparatus, the different sensory components of the composite reward 'social interaction' were investigated individually for their ability to engender CPP.

Results: If touch was limited by steel bars spaced at a distance of 2 cm and running across the whole length of a partitioning, CPP was still acquired, albeit to a lesser degree. If both rats were placed on the same side of a partitioning, rats did not develop CPP for social interaction. Thus, decreasing the available area for social interaction from 750 to 375 cm^2 prevented the acquisition of CPP to social interaction despite the fact that animals could touch each other more intensely than through the bars of the partitioning. When touch was fully restricted by a glass screen dividing the conditioning chambers, and the only sensory modalities left were visual and olfactory cues, place preference shifted to place aversion.

Conclusion: Overall, our findings indicate that the major rewarding sensory component of the composite stimulus 'social interaction' is touch (taction), with a restriction in space to socially interact in decreasing the strength of social interaction's conditioned rewarding properties.

8 Molecular Background of Reduced Mechanical Hypersensitivity in Conditional IL-6 Signal Transducer gp130 Knock-Out Mice

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Our lab has generated a conditional knock-out mouse strain lacking the signal transducer glycoprotein 130 specifically in Nav1.8 expressing sensory neurons (SNS-gp130-/-). gp130 is part of the signal transducing complex of Interleukin-6 (Il-6) and Il-6 related cytokines. Behavioural tests reveal a reduced mechanical hypersensitivity of these mice under inflammatory and neuropathic conditions. In mammals, detection of mechanical forces by the somatosensory system is performed by primary afferent neurons. Their cell bodies are located in trigeminal ganglia and dorsal root ganglia (DRG). The molecular basis underlying mechanotransduction is only beginning to be uncovered. It is thought that specific ion channels in the membrane of the sensory nerve terminals directly or indirectly open upon mechanical stimulation.

To determine alterations in the mechanosensitivity of SNS-gp130-/- neurons, we used the whole-cell voltage clamp technique to monitor mechanosensitive ionic currents while applying focal pressure to the somal membrane of cultured DRG neurons. Alternatively, calcium microfluorimetric measurements were performed. Affymetrix® gene chip analysis was used to identify deregulated genes in DRG explants of gp130fl/fl and SNS-gp130-/- mice. Differential expression of possible candidate genes was confirmed with quantitative RT-PCR.

mRNA expression assays revealed a downregulation of the mechanosensitive ion channel TRPA1 in SNS-gp130-/- vs. gp130fl/fl mice. Furthermore, a smaller proportion of neurons responded to the selective TRPA1 agonist NPPB with calcium transients in neurons from SNS-gp130-/- vs. controls.

Our data show downregulation of TRPA1 in SNS-gp130-/- mice. To test the functional expression of TRPA1 in vivo we investigate the spontaneous pain response to TRPA1 agonists injected into the hindpaw of SNS-gp130-/- mice. We hypothesise that downregulation of TRPA1 could explain the reduced mechanosensitivity of these mice under pathological conditions.

9 Sprouty2 inhibits axon outgrowth of adult DRG neurons

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Background: Sprouty2 (Spry2) is a negative feedback inhibitor of growth factor-dependent ERK-signaling in neurons. Mammals exhibit four Spry isoforms which are evolutionary conserved. Spry2 is highly expressed in sensory dorsal root ganglia (DRG). The down-regulation of Spry2 by short hairpin RNAs (shRNAs) has been shown to promote axon outgrowth in peripheral and central neuron cultures (Hausott et al., 2009). Here, we investigated the involvement of Spry2 in growth factor-dependent axon outgrowth of sensory neurons in Spry2$^{+/-}$ and Spry2$^{-/-}$ knockout mice (on a mixed genetic background of C57BL/6 and 129).

Methods: Adult sensory neurons dissociated from DRG of Spry2$^{+/-}$ mice were plated on a poly-L-lysin/laminin substrate. A significant enhancement of the length of the longest axon (maximal distance, MD) was observed as compared to wildtype (wt) littermates. After treatment for 24 hours with Nerve Growth Factor (NGF) or Fibroblast Growth Factor (FGF)-2 the parameters for total axon length (TAL), MD and number of branch points (BPs) significantly class III beta-tubulin (TuJ 1) Calcitonin Gene-Related Peptide (CGRP) and Isolectin IB4 Biotin Conjugates (IB4) lectin *in vitro*.

Results: We observed that in Spry2$^{+/-}$ mice the CGRP-positive neuron population responded to NGF with significant increases in the TAL, MD and BPs. IB4-positive neurons of Spry2$^{+/-}$ mice revealed significant axon outgrowth in the presence of FGF-2 with regard to the MD, but not for TAL and BPs. In Spry2$^{-/-}$ mice we observed differences in the TAL and in BPs. In these cultures NGF, but not FGF-2, promoted MD, TAL and BPs.

Conclusion: Taken together, the results demonstrate that Spry2$^{+/-}$ DRG neurons exhibit enhanced axonal elongation, whereas Spry2$^{-/-}$ neurons show a tendency for increased axonal branching in the presence of growth factors. These differences are probably dependent on different activity of the Ras/Raf/ERK pathway which is currently under investigation.

10 Reversal of cocaine conditioned place preference by social interaction

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A main challenge in the therapy of drug dependent individuals is to help them reactivate interest in non-drug-associated activities. Among these activities, social interaction is doubly important because treatment adherence itself depends on it. We developed a rat animal experimental model based on the conditioned place preference (CPP) paradigm in which only four 15-min episodes of social interaction with a gender- and weight-matched male conspecific (i) reversed CPP from cocaine to social interaction despite continuing cocaine training and (ii) prevented the reinstatement of cocaine CPP (Fritz et al. 2011, Addiction Biology). The reversal of CPP from cocaine to social interaction was enhanced by the sigma1 receptor antagonist BD1047 with an ED50 of 0.0036 mg/kg (i.p.) (Fritz et al. 2011, Pharmacology 87:45-48). Social interaction also reversed cocaine CPP-induced expression of the immediate-early gene zif268 in the nucleus accumbens shell, the central and basolateral amygdale and the ventral tegmental area (Fritz et al. 2011, Addiction Biology). These findings suggest that social interaction, if offered in a context that is clearly distinct from the previously drug-associated ones, may profoundly decrease the incentive salience of drug-associated contextual stimuli. In the present study, we investigated if the two subregions of the nucleus accumbens (Acb), the core (AcbC) and shell (AcbSh) would differentially affect CPP for cocaine vs social interaction. Animals were concurrently trained for CPP to cocaine and social interaction (mutually exclusive stimulus presentation during training). We are currently investigating which type of neuron in the AcbC is affected by this reversal. This project is supported by FWF grants P18787-B05, W1206-B18, and M1169-B18, and by the Verein zur Förderung der Experimentellen Psychiatrie, Psychotherapie und Pharmakologie (VEPPP).

11 Significance of the IL-6 Signal Transducer gp130 for Neuronal Regeneration

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The interleukin-6 signal transducing receptor gp130 is involved in inflammation and pain. At present, the role of IL6/gp130 interaction in injured peripheral neurons is poorly understood. Here, we have investigated the role of gp130 in the regeneration of peripheral sensory neurons using conditional knock-out mice (SNS-gp130$^{-/-}$; Andratsch et al., J. Neurosci., 2009).
The number of free nerve endings in the skin was similar in non-injured control and SNS-gp130$^{-/-}$ mice. In contrast, dorsal root ganglion (DRG) neuron cultures from SNS-gp130$^{-/-}$ mice showed significantly reduced neurite extension and numbers of neurite bearing neurons as compared to controls. Moreover, the treatment with different IL-6 type cytokines increased the neurite length in the control neurons but not in the knock-out cells. We found lower expression levels of BDNF mRNA in explants and acutely dissociated SNS-gp130$^{-/-}$ DRG neurons. However, the substitution of neurotrophins like NGF or BDNF did not rescue neurite outgrowth in SNS-gp130$^{-/-}$ cultures. Quantitative PCR indicated that the mRNA expression of neurotrophin receptors was unchanged in the knock-out neurons.
In order to investigate the downstream signaling pathways, we found that phosphorylation of ERK 1/2 was not affected by the deletion of gp130. Interestingly, the phosphorylation of STAT3 was decreased in the SNS-gp130$^{-/-}$ cultures. Treatment of the cells with leptin, which stimulates STAT3 via a different receptor, partially rescued the neurite outgrowth in a proportion of knock-out DRG neurons.
Mechanical and heat sensitivity and motor capabilities were monitored for 25 days after sciatic nerve crush injury in *vivo*. Recovery of sensitivity was similar in wild type and gp130$^{fl/fl}$ control mice but significantly delayed in SNS-gp130$^{-/-}$ mice.
Our data show that regeneration of neurons was significantly impaired in SNS-gp130$^{-/-}$ *in vitro* and *in vivo*. We therefore suggest that gp130 is an important regulator of regeneration in peripheral neurons.

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12 *N*-methyl *D*-aspartate Receptor Antibodies in Neuroinflammatory Diseases

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Background: Autoimmune diseases are characterised by the presence of antibodies (Ab) against autologous epitopes and inflammation of the tissue. Several pathogenic Ab have already been identified which target antigens within the central nervous system. Ab against the water channel aquaporin-4 (AQP4) are present exclusively in neuromyelitis optica (NMO), whereas myelin oligodendrocytes glycoprotein (MOG) is targeted in a subgroup of pediatric patients with acute disseminated encephalomyelitis (ADEM) and multiple sclerosis (MS). Recently, Ab against the *N*-methyl *D*-aspartate receptor (NMDAR) were identified in NMDAR encephalitis. The absence of specific Ab in some patients with NMO, and to a larger extend in patients with ADEM and MS, prompted a screening for NMDAR Ab of sera from patients with neuroinflammatory diseases.

Methods: A cell-based assay was developed to detect NMDAR Ab in serum and cerebrospinal fluid (CSF) of patients. HEK293A cells were transfected with three subunits required for the generation of functional NMDAR. Ab binding to native NMDAR on the surface of HEK293A cells were detected by immunofluorescence. To evaluate sensitivity and specificity, a cohort of patients with MS or other neurological diseases and healthy controls was tested for NMDAR Ab. Two patients with known NMDAR encephalitis were used as positive controls.

Results: Ab titers of sera from two NMDAR encephalitis patients were 1:1280 and 1:640, respectively. Sera from 55 MS patients, 39 patients with other neurological diseases and 46 healthy controls were negative for NMDAR Ab.

Conclusion: The data indicate high specificity and sensitivity of the cell-based assay to detect NMDAR Ab in patient's sera. Further Ab testing will also include the analysis of sera and CSF from patients with NMO and ADEM.

13 Development of a Microarray-chip for Expression Anaylsis of Neural Non-coding RNAs

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Small non-protein-coding RNAs (ncRNAs) play an important role in gene expression regulation and have been implicated in developmental processes, as well as in a number of central nervous system (CNS) specific diseases. To gain insight into regulation of gene expression by ncRNAs in the developing mouse brain, we generated three distinct cDNA libraries, obtained from ribonucleo-protein particles (RNPs). Those libraries, deriving from embryonic stem (ES) cells differentiated into neural progenitor and subsequently neuronal-glial cells, were analysed bioinformatically for differences in their expression. The most differentially expressed and most highly abundant ncRNAs are further used to design a microarray chip, for expression profiling of ncRNAs in CNS diseases.

To that end, about 26 Mio. cDNA sequences from all three libraries were subjected to high-throughput sequencing. After annotation and assembly of the sequence reads by the Automated Pipeline for Annotation of RNA Transcripts (APART), the different libraries were normalized according to the total number of reads. Moreover, an expression analysis was conducted by ranking the obtained ncRNAs regarding to their abundance and difference in expression.

As a result, we found about 100 promising novel regulatory non-coding RNAs from intergenic regions that are highly and differentially expressed. The majority of those candidates is small in size, composed of less than 26 nucleotides. Furthermore, many of already existing and annotated miRNAs were found in the dataset and their expression patterns could be verified in the literature.

The next step is to design probes from the most interesting candidates, in order to test for feasibility of expression profiling with a non-coding microarray chip.

14 Identification of Non-coding RNAs Involved In Neural Differentiation

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Non-coding RNAs are important for development, have been shown to be involved in differentiation processes and represent a significant part of the transcriptome. Non-coding transcripts, such as microRNAs and longer non-coding RNAs have been identified in mouse embryonic stem cells (mESCs), which are an important *in vitro* model system not only to study development but also organ repair and stem cell therapy. Their differentiation program has been extensively studied but is not fully elucidated and therefore it is important to further characterize it.

Most, if not all, biologically functional identified non-coding RNAs in Eukarya are known to be associated with proteins forming ribonucleo-protein (RNP) particles. In order to isolate non-coding RNAs that are likely to be functional, we have generated RNP libraries from three different stages of mESCs differentiating upon addition of FGF2.

The RNP libraries raised were high-throughput sequenced and expression analysis revealed about 1000 differentially expressed candidates mapping to intergenic and intronic regions, which could potentially be novel functional non-coding RNAs. These candidates present a size bias of 18-26 nt and seem to be important for mESCs neural differentiation since they are among the highest and most differentially expressed ncRNAs. Additionally they were found to be expressed in primary hippocampal neurons and /or astrocytes, which is a further indication towards their potential functional role.

We aim to select the most interesting non-coding RNAs for further functional analysis and study their role during neural differentiation.

15 Activation of kappa opioid receptors reduces EEG seizure activity in a mouse model of temporal lobe epilepsy

Luca Zangrandi & Christoph Schwarzer

Background: Neuropsychiatric disorders are one of the main challenges of human medicine with epilepsy as one of the most common and serious disorders of the brain. Temporal lobe epilepsy represents the most common type of epilepsies and is often accompanied by marked neuronal degeneration. One main factor that causes neural loss is the excitotoxicity of glutamate, which is copiously released during seizures and hypoxia accompanying seizures. There is evidence that endogenous opioids, namely dynorphin (Dyn), act as modulators of neuronal excitability. It was also shown that the deletion of proDyn in mice and low expression in humans is associated with increased epilepsy vulnerability. Dyn targets opioid receptors and in particular the kappa opioid receptor (KOP). The KOP in the hippocampal formation are located in very strategically points for the control of the glutamate release and most important they are not altered under epileptic conditions. Interestingly, proDyn expression is reduced after an initial increase in most epilepsy models and activation of KOP may be neuroprotective. Still, the functional background of these neuroprotective effects is not fully understood.

The aim of this study was to investigate the influence of KOP agonists on EEG patterns of epileptic mice.

Methods: Kainic acid (KA; 3nmoles in 50 nl saline) was injected unilaterally into dorsal hippocampus, causing acute and delayed behavioural and EEG effects. 4 channel EEG traces were recorded from ipsi- and controlateral hippocampi and motorcortices applying depth- and surface electrodes, respectively. The KOP specific agonist U-50488H and were dissolved in saline (adjusted to pH 7.4) and applied ip.

Results: Sharp waves and paroxysmal discharges in the ipsilateral hippocampus were recorded about 2 weeks after KA injection. Paroxysmal discharges were accompanied by behavioural arrest and stereotypic behaviour like head nodding. Application of KOP agonists at different doses (5, 20 mg/kg) markedly reduced paroxysmal discharges. These data represent observations from preliminary experiments.

Conclusions: Data collected so far confirm the anticonvulsant action of KOP agonists in the subchronical phase of epilepsy, suggesting that neuroprotective effects are indeed due to reduced seizure activity.

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16 The role of Bim and Bmf in mammary gland development and breast cancer

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During development and in tissue homeostasis cell death has an important function. Dispensable, inoperable, dangerous or harmful cells within a multicellular organism need to be removed to protect against cancer or autoimmunity. Bim and Bmf are proapoptotic members of the Bcl-2 family with tumour suppressive function. As they are reported to be involved in developmental processes of the mammary gland, we wanted to address the question if they play a functional role during involution, which is a phase of massive cell death where the lactating mammary gland is remodeled into a virgin like structure. We observed that mice, which were deficient for Bim or Bmf, show a delayed involution phenotype compared to the wild type animals.

As we noted an involvement of these two proteins in tissue remodeling of the mammary gland, we decided to look also at the tumour suppressive function of Bim and Bmf in a transgenic mouse model of breast cancer (MMTV/*neu*). If one of these proteins exerts a tumour suppressive function, we expected the tumour onset, which has a mean latency of about 205 days, to be accelerated. Interestingly, bmf-deficient animals developed the tumours as early as the wild type animals, excluding a tumour suppressive function of Bmf in this cancer model. Surprisingly, and contrary to our expectation, *bim*$^{-/-}$ mice developed the tumours significantly later. As *Bim*$^{-/-}$ animals show signs of autoimmunity, which is published on a mixed genetic background but was yet not reported in FVB mice, it remains possible that the immune status of these mice impacts on tumor development. It is now under investigation, if mice develop tumours later due to a hyperactive immune system in the absence of Bim, which could be responsible for tumour inhibition, or if *bim*$^{-/-}$ mice do show changes in differentiating epithelium or mammary stem cell subsets. Our approaches to unravel the molecular basis of this phenomenon will be discussed.

17 Bioinformatic analysis of mRNA expression profiles from children with acute lymphoblastic leukemia

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Childhood acute lymphoblastic leukemia (chALL) is a malignant, heterogeneous disease of the bone marrow where early lymphoid precursors, designated as lymphoblasts proliferate and replace normal hematopoietic cells. Children suffering from this disease are treated according to the Berlin-Frankfurt-Münster protocol initially for one week with a systemic glucocorticoid (GC) monotherapy causing massive cell death in the malignant lymphoblasts. The treatment response, however, differs widely between individual patients and different chALL subtypes.
To investigate the still unknown molecular mechanisms of GC induced apoptosis as well as the clinically relevant resistance to GCs (pre-existing or occuring during therapy), we assembled a large data set of Affymetrix-based gene expression profiles from 59 chALL patients mostly taken before and during GC treatment. In addition we measured active GC levels in the patients' plasma and collected clinically relevant parameters including results from diagnostic screens and treatment effects (decrease in lymphoblast counts). To address whether GC response and/or GC sensitivity correlates with different molecular chALL subtypes, we group the patients based on their basal gene expression profiles into different chALL subtypes (T-ALL, precursor B-ALL, BCR-ABL1, ETV-RUNX1, E2A-PBX1, MLL and hyperdiploid). Similarly, further groups are based on clinical and phenotypical information to identify possible correlations between these parameters and GC response and treatment effects.
We generated mRNA signatures that might help to develop a customized microarray or similar tools for chALL diagnosis and patient stratification. These signatures, i.e. sets of mRNAs representative for a variety of well-known tumor characteristics such as ploidy, immunological surface markers, chromosomal rearrangements, etc., created by different bioinformatic approaches, build the heart of such a diagnostic approach.

18 PDCD5 - A Novel Regulator of p53 Dependent Cell Cycle Arrest and Apoptosis in Response to DNA Damage

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In order to prevent mutations, cells have evolved various means to repair damaged DNA. Signaling pathways induced by genotoxic stress lead to arrest of the cell cycle and DNA repair. However, if repair of DNA damage is not successful, cells undergo programmed cell death (apoptosis) to eliminate damaged and potentially dangerous cells from the tissue.

One important and well-studied regulator of the decision between cell cycle arrest and apoptosis is the tumor suppressor p53. In response to genotoxic stress, p53 is stabilised and translocates to the nucleus, where it induces the transcription of several target genes involved in cell cycle arrest or apoptosis. However, what regulates the decision between the transcription of pro- and non-apoptotic target genes by p53 remains incompletely understood.

The histone acetyltransferase Tip60 has been shown to specifically acetylate p53 at Lysine120. This modification targets both Tip60 and p53 preferentially to the promotor of the pro-apoptotic Puma gene, leading to programmed cell death. The exact mechanism targeting p53 to specific promotors of cell death encoding genes in response to DNA damage, however, is still unknown.

We have identified a protein, PDCD5, which interacts with both p53 and Tip60. This interaction is restricted to the nuclear compartment and increased only in response to DNA damage, but not other death inducing stimuli. PDCD5 is a highly stable protein that is expressed in all tissues tested and localises both to the cytoplasm and the nucleus. In response to different apoptotic stimuli, we could not detect a difference in PDCD5 expression on RNA or protein level. Interestingly, PDCD5 contains a dsDNA binding domain, but initial experiments suggest that PDCD5 has no general DNA binding ability.

In conclusion, we identified a novel protein-protein interaction which might be responsible for fine tuning the decision between p53-induced cell cycle arrest and apoptosis in response to DNA damage.

19 A Novel Androgen Receptor Regulatory Loop: Posttranscriptional Androgen Receptor Regulation Through the Midline 1 Ribonuclear Protein Complex

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Background: Deregulation of the androgen receptor (AR) signaling pathway is a hallmark of prostate cancer progression to an advanced, castration-resistant tumor stage. The MID1/PP2A/ α4 complex which is a microtubule-associated ribonucleoprotein complex was shown to regulate the stability and intracellular localization of associated mRNAs, among them AR mRNA. Our aim in this study is the exploration of posttranscriptional regulation of androgen receptor (AR) protein through interaction with the midline 1/protein phosphatase 2A/Alpha4 (MID1/PP2A/α4) ribonuclear protein complex.

Materials and Methods: RNA interference (siRNA) was used to knockdown proteins in several cellular prostate cancer models. For AR activitation or inhibition synthetic androgen analog R1881 and anti-androgen Casodex, respectively, were used. AR DNA binding sites in the MID1 gene were identified in DuCaP cells by chromatin immunoprecipitation (ChIP) followed by DNA sequencing. Reporter gene vectors were constructed with putative wild type and mutated androgen responsive elements (AREs) and their functional activities were determined in reporter gene assays.

Results: Down regulation of MID1 or α4 proteins by specific siRNAs resulted in a reduced AR protein level in the AR positive cell lines. MID1 knockdown also affected proliferation and migration in both AR positive and negative prostate cancer cell lines. Androgen treatment decreased the MID1 protein as well as MID 1 mRNA level and Casodex abrogated this effect. Several AR binding sites were identified in the MID1 gene using ChIP sequencing. Their functional activities as androgen responsive elements (AREs) were analyzed and confirmed in reporter gene assays using reporter vectors with wild type and mutated AREs.

Conclusions: Our results support a negative feedback loop between AR and MID1.This is a new concept of AR protein modulation in tumors that awaits further analysis with regard to impact on therapies targeting the AR function and the progression of prostate cancer to a castration-resistant tumor stage.

Program: MCBOSupervisor: Helmut Klocker

20 LCMV-pseudotyped VSV for Oncolytic Virotherapy of Ovarian Cancer

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Background: Ovarian Cancer is one of the leading causes of death from gynecological malignancies in the western world. The prognosis of patients remains devastating since tumors are usually diagnosed at advanced stages and common therapeutic strategies such as surgery and chemotherapy reach their limit. A promising new approach is the use of vesicular stomatitis virus (VSV)-based oncolytic virotherapy as VSV is one of the most potent oncolytic viruses and there is no pre-existing immunity among the human population. However, VSV's glycoprotein-mediated inherent neurotoxicity has hindered clinical development so far.

Methods: To abrogate the VSV-inherent neurotoxicity, we pseudotyped VSV with the non-neurotropic envelope glycoprotein of the lymphocytic choriomeningitis virus (LCMV-GP), (Muik et al, J. Virol., 2011). Neurotoxicity of pseudotyped VSV(GP) was investigated upon intracranial injection in mice. Oncolytic potency was tested in ovarian cancer cell cultures and in a subcutaneous ovarian cancer xenograft mouse model.

Results: VSV(GP) exhibited a more than 10^6-fold higher LD50 compared to VSV wildtype upon intracranial injection in mouse brain. Furthermore, effective oncolytic activity of VSV(GP) could be demonstrated in ovarian cancer monolayer and spheroid cell cultures. Accordingly, intratumoral injection of VSV(GP) into subcutaneous ovarian cancer xenografts in mice led to an overall response rate of 100% tumor regression.

Conclusion: The results of our in vitro and in vivo studies demonstrate that LCMV GP-pseudotyped VSV exhibits a highly beneficial toxicity and efficacy profile. Thus, it represents an extremely promising candidate for oncolytic virotherapy of ovarian cancer.

21 Molecular Functions of NADPH-Oxidases in Prostate Carcinogenesis and Pathophysiological Processes

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Background: ROS produced by membrane-bound NADPH-oxidases are known to play crucial roles in various physiological and pathophysiological processes. Given that Nox4 is required for proper cell proliferation in healthy tissue but contributes to lesion progression in diseased tissue, it provides an attractive therapeutic target. Cumulative studies suggest a nuclear localization of Nox4. The discovery of a Nox4 splice variant lacking five of the six transmembrane domains allows the assumption of alternative functions besides ROS production.

Methods: hTERT immortalized prostate stromal cells were treated with 1ng/ml TGFβ for 24h. Transdifferentiation was quantified on protein and mRNA level by Western Blot and qRT-PCR. Nox4 siRNA and plant-derived Nox4 inhibitors were tested to reduce transdifferentiation. The truncated Nox4 splice variant (Nox4D) was cloned into a pcDNA3.1(-)hygro Vector and transfected into U2OS cells. Nuclear localization was investigated by Western Blot and Immunofluorescence and co-localization with ZSCAN18 was studied by Immunofluorescence as well.

Results: TGFβ treatment induced transdifferentiation of prostate stromal cells, as shown by increase of mRNA and protein expression of Nox4 and the differentiation markers αSMA and IGFBP3. Whereas Nox4 inhibitors dose-dependently reduced transdifferentiation, the general ROS inhibitor DPI nearly completely blocked mRNA expression of Nox4. In a yeast two hybrid screen we identified the nuclear transcription factor ZSCAN18 as potential target of Nox4. Nox4D is associated to membrane structures with distinct perinuclear localization. ZSCAN18 co-localizes with the perinuclear fraction of Nox4D.

Conclusion: Targeting Nox4 displays a convenient biological system for screening new Nox4 inhibitors. As DPI application significantly reduces Nox4 mRNA expression we suggest a positive feedback-loop between Nox4 and ROS, thus stimulating its own expression. The perinuclear co-localization of ZSCAN18 and Nox4D suggests another, maybe transcriptional regulative function than known so far.

22 Functional Analysis of Epithelial Cell Adhesion Molecule (EpCAM) Knockdown In Breast Cancer

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Background: The Epithelial Cell Adhesion Molecule (EpCAM) is a calcium-independent homophilic cell adhesion molecule which is overexpressed in variety of tumors, such as breast and colon cancer. In primary and metastatic breast cancer EpCAM gene expression has been shown to be significantly increased. Previously, it was shown also that EpCAM can modulate cell-cell contacts by antagonizing E-cadherins, therefore can change cell-cell adhesion from strong to weak. In particular, overexpression in primary breast carcinomas correlates with bad prognosis and poor overall survival in lymph node-positive breast cancer patients. All these observations suggest that EpCAM overexpression correlates with tumor invasion and metastasis, which are major hallmarks of aggressive disease progression.

Methods and results: In healthy breast tissue EpCAM is predominantly expressed on the basolateral compartment of epithelial cells. By the use of Immunohistochemistry we showed that the strict basolateral expression of EpCAM gets lost in breast cancer cells. However, strong protein expression seems to be conserved within metastasis.

Commercially available breast cancer cell lines with a strong EpCAM expression (MCF-7, SK-BR-3 and T-47D) were selected for transfection using a lentiviral system containing a shRNA to obtain a permanent downregulation. Target gene expression and gene downregulation was analyzed and confirmed by qPCR and Western Blots methods. *In vitro* cell growth was analyzed by cell counting, BrDU or Ckk8 assays. In comparison to control breast carcinoma cells, EpCAM downregulation by shRNA reduced DNA synthesis *in vitro* under 3-D culture conditions, but not under standard (2-D) culture conditions. New generated breast cancer cell lines were analyzed also *in vivo* in a chicken tumor xenograft model to study tumor growth, invasion and angiogenesis.

Conclusion: We suggest that a complex 3-D environment is necessary for functional EpCAM studies.

Tumor xenografts with a high EpCAM expression were bigger in size and more invasive to the host tissue. All these investigations will help us to gain to better understanding of the role of EpCAM in tumor biology and bring more light into its function.

23 Phosphorylation of $p27^{Kip1}$ by the class III receptor tyrosine kinase FLT3

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The Cdk inhibitor p27 plays a central role in cell cycle control by inactivating cyclin dependent kinases (Cdks). The level of p27 protein expression is mainly regulated by posttranslational mechanisms. Recently we described the modification of p27 by Y88 phosphorylation. This results in an impaired Cdk inhibition and the partially active cyclin/Cdk2/p27 complex can phosphorylate T187 of p27, which is essential for the SCF-Skp2 mediated ubiquitin-dependent proteasomal degradation of the inhibitor.
The oncogenic non-receptor tyrosine kinases Bcr-Abl, Lyn, Src and Jak2 are known to phosphorylate p27. We found that the receptor tyrosine kinase Flt3 (Fms like tyrosine kinase) can induce p27 phosphoryation when coexpressed in 293T cells. Activating mutations of Flt3, such as the internal tandem duplication (ITD), are the most common genetic lesions in acute myeloid leukaemia (AML).
Using phospho-Y88-specific antibodies, we identified Y88 of p27 as the main target site of Flt3 and Flt3-ITD in transfection assays, which could be confirmed with a p27-Y88F mutant. Activation of endogenous wild-type Flt3 in the human leukemic cell line THP-1 by Flt3 ligand also leads to Y88-phosphorylation of p27. Endogenous p27 is phosphorylated on in the Flt3-ITD mutant expressing human acute monocytic leukemia cell line MV4;11. This phosphorylation is suppressed by the Flt3 inhibitor SU5614. The phosphorylation of p27 could be direct, as Y88-phosphorylation was only suppressed by SU5614, but not by inhibitors of the Flt3-ITD activated downstream kinases cSrc or Jak2. A potential direct phosphorylation of p27 by Flt3 will be further investigated *in vitro* using recombinant purified GST-Flt3 and p27 as a substrate.
Co-immunoprecipitation experiments of transfected p27 and Flt3-ITD suggest that they can be found within one protein complex. Deletion experiments within the cytoplasmic domain of Flt3 indicate that the tyrosine kinase domain-1 is required for p27 binding.
To further investigate the *in vivo* relevance of this p27-Y88 phosphorylation by Flt3, we plan to investigate AML patient samples for p27-Y88 phosphorylation and to analyse the relevance of the phosphorylation in an Flt3-ITD tumour model using knock-in mice where Y88 of p27 is mutated to non-phosphorylatable phenylalanine.

24 The Cold-Inducible RNA-Binding Protein CIRP Binds $p27^{kip1}$ mRNA and Strongly Upregulates $p27^{kip1}$ Expression

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Background: $p27^{kip1}$ is a member of the Cip/Kip family of cyclin-dependent kinase inhibitors that plays a fundamental role in cell cycle control. To better understand the network controlling $p27^{kip1}$ synthesis, we investigate novel $p27^{kip1}$ 5' UTR binding factors which might regulate $p27^{kip1}$ translation.

Methods: By applying "Northwestern" procedure to protein arrays we identified the cold-inducible RNA-binding protein CIRP as a novel $p27^{kip1}$ 5' UTR binding factor. Protein-mRNA immunoprecipitations confirmed that CIRP binds the $p27^{kip1}$ mRNA and reporter assays showed that CIRP regulates the activity of the 5' and 3' UTR of $p27^{kip1}$ transcript. CIRP was first described as a protein which is strongly expressed by moderate hypothermic conditions and plays a role in the metabolic response to cold stress. We hypothesized that CIRP might trigger cold-induced suppression of cell growth by regulating $p27^{kip1}$ expression. We did temperature-shift experiments and immunoblotting to analyse protein expression. Transfection experiments were done in order to overexpress CIRP or downregulate it via RNA interference; the effects on the cell cycle were analysed by flow cytometry.

Results: Since we observed that CIRP binds $p27^{kip1}$ mRNA, we investigated if and under which conditions CIRP affects $p27^{kip1}$ protein expression. We observed that only in few cell lines CIRP levels rise in mild hypothermic conditions, and that this correlates with a higher expression of $p27^{kip1}$. Moreover, silencing CIRP expression leads to a reduction of $p27^{kip1}$ protein levels. Independently of the temperature, CIRP overexpression causes a significant upregulation of $p27^{kip1}$ and induces accumulation of cells in the G1 phase of the cell cycle. We currently determine the molecular mechanism that leads to this strong induction of $p27^{kip1}$ and the role of the two $p27^{kip1}$ untranslated regions in this regulation.

Conclusion: CIRP induces $p27^{kip1}$ expression and directly binds to the $p27^{kip1}$ mRNA in vitro and in vivo. We hypothesize that it regulates $p27^{kip1}$ translation via interaction with the UTRs of $p27^{kip}$ mRNA. Elucidating the role of CIRP could help to identify a new signaling network involved in the regulation of $p27^{kip1}$ expression and possibly in disorders originating from deregulated expression of the $p27^{kip1}$ protein.

25 Expression of eIF3a in Gallbladder Carcinoma (GBC) and Urinary Bladder Carcinoma (UBC)

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Background: GBC and UBC are common, aggressive tumors with high mortality rates. Both entities are not completely understood in terms of their etiology and genetics. eIF3a, the largest subunit of eukaryotic initiation complex eIF3, is up-regulated in many cancers and its suppression leads to inhibition of tumour cell proliferation *in vitro.* Our aim is to evaluate eIF3a in GBC as well as in UBC to gain insight in the pathogenesis of these tumors and to further determine the role of eIF3a in cancer development and progression.

Methods: We investigated expression of eIF3a in 74 GBC and 178 UBC cases, by IHC, questioning its potential as prognostic GBC/UBC marker. For GBC we compared eIF3a expression with survival data. Protein expression levels of eIF3a were analysed in three GBC and five UBC cell lines by western blotting. Furthermore by manipulating eIF3a levels in tumor cell lines we want to explore whether eIF3a expression levels can directly influence cell cycle progression and translation (canonical and alternative pathways). We are therefore generating an inducible eIF3a knockdown construct.

Results: eIF3a is upregulated in UBC and GBC when compared to normal tissues. Higher intensity scores of GBC display a lower 5 year survival rate when compared to GBC with lower intensity.

We have successfully tested two lentiviral shRNA constructs for the inducible knockdown of eIF3a. The knockdown construct generated proves efficient in all tested cell lines and first results show an association of eIF3a knockdown with growth retardation of tumor cells.

Conclusions: Overexpression of eIF3a in GBC is an indicator of a more aggressive GBC type with lower 5-year survival rate. GBC/UBC cell lines provide a model for studying the mechanistic role of eIF3a in cancer progression. The knockdown of eIF3a leads to reduced proliferation rates, indicative of subcellular changes airising probably not exclusively from altered translational profiles.

26 Functional Analysis of Caspase-2 Mutants

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Caspases are proteases involved in various biological pathways, including inflammation, proliferation and programmed cell death. Within the family of caspases, caspase-2 (C2) is highly conserved across species and the only caspase that can be detected in the nucleus. Despite several reports showing involvement of C2 in cell death in response to different treatments, more recent studies using C2-deficient mice and cells contradict those findings. Apart from a possible role in apoptosis, some findings also suggest C2 involvement in non-apoptotic processes triggered in response to DNA damage like cell cycle arrest and DNA repair.

Structurally, C2 can be grouped within the family of initiator caspases that become activated through dimerization, which is proceeded by several processing steps. However, C2 is not capable of cleaving effector caspases, a common feature of initiator caspases. Since no specific C2 substrates are known and due to the lack of an obvious phenotype of C2-deficient mice, the precise biological role of C2 remains enigmatic.

To further clarify activation, function and regulation of C2, several C2 mutants defective in catalytic activity, processing, localisation and posttranslational modifications were generated. These C2-mutants were used to reconstitute $C2^{-/-}$ mouse embryonic fibroblasts (MEF) under the regulation of the Tet On Advanced Inducible Gene Expression System. Reconstituted $C2^{-/-}$ MEF clones were analysed for the impact of those mutations on apoptosis induced by various treatments, cell cycle arrest in response to irradiation and proliferation. In addition, processing and localisation of C2 in response to DNA damage was investigated.

Our results showed no significant difference among cells expressing different C2 variants in response to apoptosis inducing treatments. Furthermore, proliferation was not altered by overexpression of C2 or of C2 mutants, rendering the protease dispensable for these processes, at least in MEF.

27 Cbl-b is a critical Regulator of the Natural Killer (NK) Cell Activation Threshold

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Background: NK cells play a critical role for cancer immune surveillance, and thus are highly attractive immune cells for adoptive cancer therapies. The E3 ubiquitin ligase Cbl-b acts as a threshold regulator. As a consequence, T cells lacking Cbl-b are hyperactive and do not need a co-stimulatory signal for activation. We here investigate whether Cbl-b expression also affects the NK cell compartment.

Methods: Expression of Cbl-b in highly FACS-sorted murine NK cells was analyzed by western blotting and real time PCR. The functional role of Cbl-b in NK cells was tested using either highly purified NK cells from *cblb*-deficient or wt mice or by knocking down Cbl-b in purified wt NK cells. Moreover, the role of NK cells in the tumor rejecting phenotype of *cblb*-deficient mice was defined by NK depletion experiments.

Results: Our results demonstrate that highly purified human and murine NK cells express high levels of Cbl-b mRNA and protein. We next tested the *in vitro* function of murine and human NK cells. $CD3^-$ $NK1.1^+$-sorted NK cells from *cblb*-deficient animals are hyperactive as shown by increased cytotoxic activity and cytokine production as compared to NK cells sorted from wt littermates. Accordingly, knockdown of *cblb* by means of siRNA in a human NK cell line (NKL) or in primary murine NK cells also increases their cytokine production as well as their cytotoxic potential. Moreover, *cblb*-deficient NK cells are resistant towards the suppressive effects of TGF-ß and Tregs. We can also demonstrate that the depletion of NK cells in *cblb*–deficient animals totally abrogates the tumor- resistance phenotype of *cblb*-deficient animals.

Conclusion: We here provide *in vitro* and *in vivo* evidence that Cbl-b is an intrinsic negative regulator of NK cell activation counteracting TGF-ß and Treg-mediated immune-suppression. This study sets the stage for optimization strategies of NK-based adoptive immune cell transfer protocols for cancer treatment.

28 Investigating the Role of BH3-only Proteins in B Cell Development

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B cells undergo many selection processes before becoming mature and immunocompetent. The TNF-family ligand B cell-activating factor (BAFF), which binds three receptors: BCMA, TACI and BAFF-R, plays an important role in B cell development and survival. Its absence causes the loss of most mature B cells including transitional type 2 (T2), follicular (FO) and marginal zone (MZ) B cells. This deficit can be partially rescued by overexpression of Bcl2, the founding member of the Bcl2 family of proteins. The survival function of Bcl2 is antagonized by BH3-only proteins, pro-apoptotic members within the same family, such as Bim or Bmf. Similar to overexpression of Bcl2, high-levels of BAFF lead to autoimmune disorders due to the survival of autoreactive B lymphocytes, whereas BAFF loss results in B cell death. Consistently, loss of Bim or Bmf causes lymphadenopathy in mice. Elevated levels of BAFF as well as loss of BH3-only proteins have also been observed in patients suffering from autoimmunity and certain forms of cancer, suggesting a tight connection between BAFF signaling, BH3-only proteins, B cell survival and pathology in mice and men.
Here, we aim to understand to what extent the biological effects of BAFF are based on the modulation of proapoptotic factors such as Bim or Bmf. Therefore, we crossed *bim*$^{-/-}$ and *bmf*$^{-/-}$ animals with mice that overexpress a TACI-Ig fusion protein, in which BAFF is sequestered and non-functional.
Preliminary results suggest, that the deletion of Bim or Bmf can restore in part the survival of T2, FO and MZ B cells in TACI Ig transgenic mice, which is even more pronounced when both BH3-only proteins are lacking.
We conclude that BAFF acts by modulating the expression and/or function of Bim and Bmf, but the molecular basis and if the surviving B cells are also functional remains to be investigated. Based on these findings we want to define whether well-known cell death regulators of the Bcl-2 family, that are already validated drug-targets, can be used in the treatment of autoimmunity and cancer that associate with deregulated levels of BAFF.

29 Disassembling the ESCRT-III membrane scission machinery

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The down-regulation of most if not all transmembrane proteins requires the activity of the evolutionary conserved endosomal sorting complex required for transport (ESCRT) proteins. The ESCRT machinery is essential for the specific recognition and sorting of ubiquitinated transmembrane proteins into the intraluminal vesicles of multivesicular bodies (MVBs), which is a prerequisite for their lysosomal degradation. During MVB sorting, the four core complexes ESCRT-0, -I, -II and –III together with the AAA$^+$-ATPase Vps4 cooperate to direct sorting of cargo and inward budding of the endosomal membrane. The ordered assembly of four ESCRT-III subunits (Vps20→Snf7→Vps24→Vps2) and probably the subsequent Vps4 dependent ESCRT-III recycling mediate the late steps in the ESCRT reaction, membrane deformation and scission. *In vivo* the disassembly of ESCRT-III is a highly regulated reaction in which the ESCRT-III MIMs (MIT-interacting motifs) and at least 4 accessory factors coordinate Vps4 activity.

While ESCRT-III disassembly is required to recycle individual ESCRT-III subunits into the cytoplasm (and thereby prevent entry into the MVB vesicle) the exact molecular mechanism of this reaction is unclear. We aim to dissect the order of ESCRT-III subunit release and we investigate the role of the Vps4 MIT-MIM interactions in the disassembly process. Further we want to address whether Vps4 catalyzed ESCRT-III disassembly is actively involved in shaping a nascent bud or just serves to recycle ESCRT-III subunits back into the cytoplasm.

ESCRT-III disassembly is initiated by binding of Vps4 to the ESCRT-III subunit Vps2 in a co-factor supported recruitment process. Our data suggest that, once activated, Vps4 disassembles the ESCRT-III complex in an ordered manner starting from the Vps2 end of the complex. During the disassembly process, Vps4 is guided by interactions with the ESCRT-III core subunits. The disassembly of the Snf7 filament thereby might play a role in regulating the MVB luminal vesicle size. Our studies might give better insight into the molecular mechanism of ESCRT-III disassembly and could help to reveal Vps4´s role in membrane remodelling.

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30 Characterisation of Renal Epithelial Monolayer Maturation Processes

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The ability of the kidney to concentrate, metabolise and secret compounds underlies its susceptibility to xenobiotics. The proximal tubule epithelium is one of the most susceptible regions of the nephron. Even minor disturbances in proximal tubule function could have serious consequences for homeostasis. Injury to the epithelial cells releases cell to cell contacts and promotes proliferation and tissue repair. In order to better understand these processes, we analysed temporal transcriptomic alterations of human renal proximal tubule cultures during monolayer formation.
Primary human proximal tubule cells and the recently developed human RPTEC/TERT1 cell line were seeded at ~ 30 % confluence. Cell cultures were maintained in hormonally defined DMEM/F12 and fed every day for 16 days. At day 1 and then every third day, cultures were harvested for RNA isolation and cell cycle analysis. In addition, supernatants were collected to analyse glycolysis rates. Using Illumina HT-12 whole-genome expression arrays 1238 temporally differently expressed genes were identified.
The time of culture had a large impact on the gene expression stabilising around day 13 after seeding, concomitant with retardation in G1/0 cell cycle phase. Temporal increases in cell adhesion and tight junction proteins were observed. Additionally, subunits of Na,K-ATPase, an important primary active transporter in proximal tubules, were differently expressed during monolayer formation. Downgraded glycolysis was accompanied by alterations in energy metabolism genes involved in glycolysis, TCA, oxidative phosphorylation or fatty acid metabolism. Interestingly, oxygen consumption was higher in proliferating cells than in matured cells, but matured cells exhibited a higher oxidative capacity.
The generated data set will be useful to identify mechanistically linked biomarkers of epithelial cell injury processes.

31 Targeting Intracellular Signaling Pathways for the Prevention of Ischemia/Reperfusion-Induced Damage During Solid Organ Transplantation

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Background: Excessive production of reactive oxygen species (ROS) is a major contributor to the development of ischemia-reperfusion injury (IRI) in the course of solid organ transplantation. In particular mitochondria-derived ROS are critical for the initiation and progression of IRI, which restricts the pool of donor organs and results in elaborate follow up treatments. In various *in vivo* (IR) and *in vitro* (hypoxia/reoxygenation, HR) models we observed a consistent pattern in the activation of key intracellular signaling pathways. Most strikingly the use of p38-specific inhibitors prevented mitochondrial ROS production and cell death. Here we further dissected the contribution of p38 to IR- and HR-induced damage and provide first evidence for a therapeutic benefit of p38 inhibition *in vivo*.

Methods: Kidney transplantation and kidney clamping in the rat were used for the induction of IRI. Intracellular signaling was monitored by using phosphorylation-specific antibodies. ROS/NOS-induced tissue damage was visualized by 3-nitrotyrosine specific antibodies. To assess acute kidney injury (AKI) HSP70 expression was monitored by immunoblotting, serum creatinine and urea were measured in the clinical routine lab, and serum cystatin c and NGAL concentrations were determined by ELISA. Expression of cytokines and oxidative stress markers was measured by real time qPCR. Apoptosis of cells during IRI was assessed by TUNEL assay.

Results: Reperfusion following kidney clamping or transplantation was marked by a profound increase in the activity of p38 and the putative effector MK2 which was significantly prevented by the p38 inhbitor BIRB-796. This treatment also prevented deterioration of kidney function following IR based on reduced serum creatinine, urea, cystatin c and NGAL levels. P38 inhibition also protected from oxidative damage and significantly reduced the percentage of apoptotic cells and the expression of TNFα and HO-1 during IR. Thus the inhibition of p38 prevents key processes, which are essential for the development of IRI.

Conclusion: Inhibiting p38 signaling during IR and HR may provide a potent strategy for limiting IRI.

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32 Regions of the DHPRβ_{1a} Subunit Responsible for DHPR Voltage-Sensing in Skeletal Muscle Excitation-Contraction Coupling

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Background: In the DHPRβ_1-null zebrafish strain *relaxed* the lack of β_{1a} results in reduced DHPRα_{1S} membrane expression, in impediment of tetrad formation, and also in the elimination of α_{1S} charge movement (*Schredelseker* et al., 2005, PNAS). Recently we postulated a model describing the β_{1a} subunit as an allosteric modifier of proper α_{1S} conformation (*Schredelseker/Dayal* et al., 2009, JBC) and thus enabling full DHPR functionality in skeletal muscle excitation-contraction (EC) coupling. Here we investigate the distinct regions of β_{1a} which might be responsible for inducing the voltage-sensing function of the DHPR.

Methods: We expressed different β isoforms and chimeras in isolated myotubes of *relaxed* larvae. Quantitative immunocytochemical analysis was performed in these *relaxed* myotubes to study the level of channel expression in the membrane. To investigate the functionality of the expressed channels in the membrane, whole cell patch clamp (charge movement) analysis was performed in the *relaxed* myotubes.

Results: Quantitative immunocytochemical analyses showed that all four β isoforms (β_1-β_4) were able to fully target α_{1S} into triads. Interestingly, despite full triad targeting, β_3 was unable to restore considerable charge movement (Q_{max}, 2.53 ± 0.50 nC/μF) in contrast to the other β isoforms (Q_{max}, 8.86 ± 0.93 to 9.94 ± 2.06 nC/μF) upon expression in *relaxed* myotubes. Systematic exchanges of variable regions and conserved domains of β_{1a} with corresponding β_3 sequences revealed significantly reduced Q_{max} restoration with SH3 and C-terminal chimeras (Q_{max}, 4.02 ± 0.28 and 5.57 ± 0.74 nC/μF, respectively). In contrast, β_{1a}/β_3 chimeras with the N-terminus, HOOK and GK domain exchanged showed complete restoration of charge movement. Further investigation by systematic exchanges of the SH3 domain and the C-terminus of β_3 with corresponding β_{1a} domains showed neither the SH3 domain nor the C-terminus alone is able to restore the charge movement but β_3/β_{1a} (SH3, C) double chimera where the SH3 domain and the C-terminus of β_3 was exchanged together with respective domains of β_{1a} showed full Q_{max} restoration (Q_{max}, 8,63 ± 1,02 nC/μF).

Conclusion: Together, our data suggest a cooperative effect of the conserved SH3 domain and the variable C-terminus of β_{1a} is essential for the induction of the voltage-sensing function of the DHPRα_{1S} in skeletal muscle EC coupling.

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33 YPT53 is a Key Regulator of a Potential Membrane Stress Response Pathway

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Four major pathways target proteins for degradation, the ubiqutin-proteasome system (UPS), the ER associated degradation (ERAD), the autophagy- and the multivesicular body (MVB) - pathway. The MVB pathway is required for the degradation of most if not all membrane proteins. Membrane Protein degradation via the MVB pathway is mediated by the endosomal sorting complex required for transport (ESCRT). Upon loss of ESCRT function, membrane proteins are no longer degraded but instead accumulate inside the cell in one or two so-called class E compartments.

Our goal is to understand how cells respond to the intracellular accumulation of membrane proteins upon loss of ESCRT function. Therefore we analyzed changes in the gene expression and the proteome of ESCRT mutant cells. These analyses demonstrated that the mRNA of 124 genes was differentially regulated. Up-regulated mRNA of genes in the ESCRT mutant cells are involved in stress response and membrane transport. To address how the up-regulated genes would contribute to a membrane stress response, we performed synthetic genetic interaction studies. Of ten candidate genes only YPT53 displayed a synthetic sick phenotype in an ESCRT mutant (□*vps4*) background. □*ypt53,* □*vps4* double mutant cells grow slowly and are temperature sensitive, suggesting that somehow YPT53 contributes to survival of ESCRT mutant cells. YPT53 is an orthologue of human Rab5, a ras-like small GTPases that is a master regulator of endosomal transport. Similar to the Rab5A, B, C in higher eukaryotes, YPT53 has two homologues, Vps21/YPT51 and YPT52. Yet only YPT53 was transcriptionally upregulated. Importantly, we found that YPT53 is specifically required to control the morphology of the class E compartment, suggesting that the proper generation of a class E compartment helps cells to deal with the accumulation.
Taken together, it appears that ESCRT mutant cells activate a membrane stress response and the Rab5 orthologue YPT53 might play a key role in mediating this response by controlling the biogenesis of the class E compartment.

34 Probing protein interactions in the skeletal muscle dihydropyridine receptor complex using FRAP analysis

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Background: Skeletal muscle EC-coupling requires direct interactions of the dihydropyridine receptor (DHPR) with the ryanodine receptor (RyR1) and its organization in tetrads opposite RyR1. Accordingly, tetrad formation and skeletal muscle EC-coupling fail in myotubes expressing the cardiac α_{1C} subunit or lacking the β_{1a} subunit. This indicates that tissue-specific protein-protein interactions between α_{1S}, β_{1a} and RyR1 are important for the structure and function of the skeletal muscle EC-coupling apparatus.

Methods: Here we apply fluorescence recovery after photobleaching (FRAP) in dysgenic (α_{1S}-/-) myotubes reconstituted with GFP-tagged DHPR subunits to probe the importance of these interactions in stabilizing them in the triad.

Results: Consistent with its anchoring in the triad, the α_{1S} subunit is essentially immobile. Surprisingly, also the cardiac α_{1C} subunit, which does not interact with RyR1, is immobile. Thus, the II-III loop – RyR1 interaction is not the determing factor for stabilizing α_{1S} in the triad. When coexpressed with α_{1S}, the β_{1a} subunit showed a higher fluorescence recovery than the α_1 subunits, suggesting that it can be dynamically exchanged in the complex. Again, the recovery did not increase when β_{1a} was coexpressed with α_{1C}, indicating that its putative interaction with RyR1 does not contribute to the stability of β_{1a} in the complex. Previously we showed that all β isoforms can associate with α_{1S} and α_{1C} in the triads. When the non-muscle β_{2a} or β_{4b} subunits were expressed with α_{1S} in dysgenic myotubes their recovery after photobleaching was significantly higher than that of β_{1a}, demonstrating that the homologous β subunit binds the channel complex with higher affinity than heterologous β subunits.

Conclusions: From our analysis we conclude that the mobility/stability of DHPR subunits in the triad is independent of their organization in tetrads opposite the RyR1. Moreover we find that β subunits can dynamically exchange with the α_1 subunits and that the rate of this equilibrium depends on the affinity of the specific α_{1S} – β pair.

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35 Targeting BAG-1 Protein Interactions to inhibit Tumor Growth

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Background: BAG-1 is a multifunctional protein, which regulates cell growth, survival, intracellular signaling, and protein folding. Important for its function is the ability to associate with a variety of different proteins. These interactions are mainly mediated by the C-terminal „BAG-domain“ comprising approx. 100 amino acids. Critical interaction partners include the survival protein Bcl-2, heat shock protein 70 (Hsp70/Hsc70) and RAF kinase, the latter being frequently involved in human tumorigenesis with mutations present in approx. 10% of all human tumors. BAG-1 expression is frequently altered in human cancer and may have prognostic value. Also the depletion of BAG-1 negatively affected growth and survival of several cancer cell lines and prevented transformation by activated RAF *in vivo* and *in vitro*. Hsp70 is present at elevated levels in many cancers and promotes tumor growth. Recently first evidence has been obtained that disrupting the protein complex BAG-1/Hsp70 and to a lesser degree of BAG-1/RAF by Thioflavin S abolishes transformation *in vitro*. However, Thioflavin S is a mixture of reaction products unsuitable for lead development. **Methods:** We established a purification protocol that enabled the isolation of highly pure Thioflavin-S constituent Thio-2. Extensive characterization was performed to elucidate Thio-2's effects on growth inhibition, apoptosis induction, protein-protein interactions and MAPK signaling. **Results:** Our work resulted in the development of an inhibitor of protein-protein for BAG-1Hsc70 and BAG-/RAF (?), respectively, with the ability to suppress signaling via the MAPK pathway. This in turn negatively affected cell growth and induced apoptosis in MCF-7 cells. **Conclusion:** Given the frequent involvement of BAG-1 protein interactions in human tumors and the critical effector function of BAG-1 in RAF transformation, targeting BAG-1-mediated interactions will have therapeutic benefits in the treatment of such tumors.

36 Nuclear targeting of the calcium channel β4b subunit in wildtype and lethargic cultured cerebellar granule cells

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Voltage-gated calcium channels (VGCC) mediate calcium influx in response to membrane depolarization and regulate numerous cellular functions. Auxiliary β subunits are critical determinants of membrane expression and gating properties of VGCCs. Four distinct β subunit isoforms have been identified, all of which are expressed in brain. In our previous work we discovered the localization of the neuronal β4 subunit in nuclei of cerebellar granule cells and Purkinje cells (Subramanyam et al., 2009, Channels). Moreover, this unexpected finding was corroborated by heterologous expression of β4b-V5 in dysgenic skeletal myotubes and primary cultured hippocampal neurons.
Here we established and characterized cultured cerebellar granule cells (CGCs) from wild type and □4-null (lethargic) mice to further analyze the function of β4 nuclear targeting. CGCs were isolated from whole cerebella of six days old BALB/C mice and were allowed to differentiate in culture for 7-9 days. Immunofluorescence labeling with anti-tau and anti-MAP2 indicated elaborate axonal networks and few short dendrites, respectively. Observing calcium transients with the fluorescent calcium indicator Fluo4-AM demonstrated the spontaneous activity of the differentiated CGC cultures. Immunolabeling with synapsin and vGlUT1 revealed a high density of presynaptic specializations. However the great majority of these did not colocalize with the postsynaptic proteins PSD95, NMDA receptor and GABAA receptor, indicating that CGCs in culture very rarely form synapses. Nevertheless, depolarization-induced uptake and release of FM 1-43 dye demonstrated that the presynaptic specializations correspond to functional nerve terminals. Double immunofluorescence labeling further demonstrated the localization of calcium channel CaV1.2 and CaV2.1 subunits as well as β1 and β4 subunits in clusters on the soma, the dendrites, and along the axons. Whereas the available □4 antibody was inefficient in demonstrating the nuclear localization of endogenous or heterologous □4 subunits, nuclear targeting was revealed by labeling V5- and GFP-tagged □4b expressed in wild type and lethargic CGCs. Reconstitution of lethargic CGCs with either □4a or □4b showed isoform-specific differences in nuclear targeting. Depolarization or blocking spontaneous activity with TTX decreased or increased nuclear targeting, respectively. Thus, the properties of β4 nuclear targeting that have previously been shown in heterologous cells could now be reproduced in the native neuronal system.
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37 Characterization of Two Novel Proteins Interacting with p14/MP1 Scaffold Complex

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Backround: Cell signaling is coordinated by scaffold and adaptor proteins which organize the physical interaction of components of signaling complexes in space and time. We have shown previously that the p14/MP1 scaffold complex is localized to late endosomes [1-3] and facilitates MAP kinase signal transduction on this specific subcellular location [4-5]. It was shown recently that the p14/MP1 forms a complex with endosomal anchoring protein, called p18 [6,7], and is also essential for mTORC1 kinase signaling from late endosomes [8].

Methods: Tandem Affinity Purification (TAP), Immunofluoresce microscopy, Western blot analysis

Results and Conclusions: Using Tandem Affinity Purification (TAP) approach we have identified several MP1/p14 interacting proteins. Here we characterize two novel proteins forming a complex with p14/MP1/p18. These proteins were shown to localize to late endosomes using immunofluoresce microscopy. Physical interaction of the proteins with p14/MP1/p18 was confirmed with reciprocal TAP. Both novel p14/MP1 interacting proteins were predicted to belong to the same as p14 and MP1 Roadblock/LC7 protein family with a characteristic profilin-like fold structure [4]. Modeling revealed high similarity of novel proteins to published 3D structure of p14 and MP1. Immunofluoresce microscopy and Western blot analysis confirmed that interaction of these novel interacting proteins with endosomal membranes is p14-dependent.

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38 Thr206 and Ser213 in $p66^{SHC}$ regulate $p66^{SHC}$-induced ROS and apoptosis in response to oxidative stress

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Background: $p66^{SHC-/-}$ mice showed an about 30% increase in lifespan which correlated with an increased resistance towards oxidative stress. This elevated stress tolerance of $p66^{SHC-/-}$ mice was due to a decreased production of reactive oxygen species (ROS). It has been reported that $p66^{SHC}$ directly produces mitochondrial hydrogen peroxide through the oxidation of cytochrome *c* in the electron transport chain. PKCβ has been implicated in the activation of $p66^{SHC}$ through phosphorylation of Ser36 which is also a target for several other kinases after different stress stimuli.

Methods: As cellular models we used immortalized mouse embryonic fibroblasts (MEFs) from $p66^{SHC-/-}$ or $PKC\beta^{-/-}$ mice. Cells were stained for ROS using reduced MitoTracker Red, detected with standard microscopy, or 2‘,7‘-dichlorofluorescin diacetate (DCF-DA), measured with FACS. Ca^{2+} levels were assessed by staining with Rhod-2 AM using confocal microscopy. For survival assays cells were stained with Annexin V/PI and analyzed by FACS. Hydrogen peroxide (H_2O_2) and tert-butyl hydroperoxide (t-BHP) were used as pro-oxidants. Gö6976 is a selective PKCα/β inhibitor.

Results: We showed that MEFs from $PKC\beta^{-/-}$ mice displayed a ROS-deficient phenotype and increased resistance towards pro-oxidants comparable to $p66^{SHC-/-}$ MEFs. As Ser36 was also phosphorylated in $PKC\beta^{-/-}$ MEFs, we scanned for potential PKC phosphorylation sites in $p66^{SHC}$ and identified three other phospho-sites. $p66^{SHC-/-}$ MEFs expressing $p66^{SHC}$ mutated in Ser213 showed a decreased ROS production and were less sensitive towards pro-oxidant treatment. Furthermore mutation of Thr206 and/or Ser213 interfered with binding of $p66^{SHC}$ to Pin1, needed for translocation of $p66^{SHC}$ to mitochondria upon cellular stress.

Conclusion: Thr206 and Ser213 in $p66^{SHC}$ are critical PKCβ phosphorylation sites regulating $p66^{SHC}$ induced ROS and apoptosis in response to oxidative stress.

39 Modulatory interactions with the I-II linker of L-type calcium channels

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Calcium influx through L-type voltage-gated calcium channels (LTCCs) underlies important physiological processes like muscle contraction, neuronal excitability, modulation of gene expression and release of hormones. In the multisubunit complex of a LTCC, the β-subunit binds with high affinity to the cytoplasmic I-II loop of the pore forming α1-subunit and thereby promotes plasma membrane targeting and modulates channel gating. We have recently discovered that the Cav1.3 I-II loop peptide is targeted to the plasma membrane without β-subunit and also in complex with β-subunits and other proteins (β, β+ RIM) [Gebhart et al., Mol. Neurosci. 44:246; 2010].
The aim of this study is to identify potential binding partner(s) of LTCC I-II loop in the plasma membrane and localize structural features of LTCC I-II loops involved in channel function and/or plasma membrane targeting. We produced FLAG-tagged I-II loops from different L-type (Cav1.1, Cav1.2, Cav1.3 and Cav1.4) calcium channels, transiently expressed them in HEK-293 cells and monitored their subcellular localization by high resolution immunofluorescence microscopy. All L-type I-II loops were localized in the plasma membrane. As expected, β-subunits (β3, non-palmitoylated β2) were evenly distributed in the cytoplasm but were targeted to the plasma membrane by co-expressed L-type I-II loops. Mutation of a single residue (W441A) in the β-subunit binding motif of the I-II loop disrupted β-subunit interaction but not plasma membrane targeting. This shows that independent structural motifs determine β-subunit binding and targeting of LTCC I-II loops. Deletion of amino acids 91-119 on the I-II loop prevented plasma membrane targeting suggesting that this region contains structural motifs required for plasma membrane targeting. This region is predicted (PSIPRED) to form an alpha helix with positive charges located predomionantly on one side of the helix thus forming a possible interaction site for binding partners. We propose that this region is either involved in a protein-protein interaction or lipid-protein interaction that underlies the binding of the loop to the plasma membrane. This prediction is currently investigated using site-directed mutagenesis.

Support: FWF (P20670, W11, University of Innsbruck)

40 Survival Signaling Pathways and Reactive Oxygen Species in the Control of Cell Death

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Background: Prolonged growth factor (GF) removal leads to the induction of apoptotis. Survival kinases like RAF and AKT or antiapoptotic members of the Bcl-2 protein family can delay cell death under these conditions. We identified deregulation of mitochondrial ROS and Ca^{2+} as essential intermediates in cell death induction following GF removal in 32D cells, which was suppressed by activated RAF, AKT or the overexpression of Bcl-2. Others demonstrated the requirement to inactivate Mcl-1 via an AKT-dependent pathway under the same death inducing conditions. The experiments presented here aimed at understanding a potential crosstalk between ROS and different signaling pathways in life and death decision.

Methods: Interleukin-3 (IL-3)-dependent parental 32D cells or 32D cells expressing constitutively active and 4-hydroxytamoxifen (OHT)-inducible forms of RAF, NIH 3T3 cells *wt* or carrying activated C-, B-RAF, or AKT were used in GF abrogation experiments. Cell viability was assessed by Annexin V/PI staining followed by flow cytometry. Alterations in mitochondrial membrane potential were detected by TMRM staining and analyzed by flow cytometry. The active form of Bax was immunoprecipitated with the conformation-specific Bax 6A7 antibody. Bax translocation and cytochrome *c* distribution were monitored following subcellular fractionation.

Results: Growth factor deprivation resulted in the loss of Mcl-1 protein in parental but not in cells protected by RAF or cells treated with the antioxidant *N-acetylcysteine* (NAC). Upregulation of Bim was detected after GF removal in NIH 3T3 and 32D cells. Activated RAF prevented conformational changes required for Bax activation. Treatment with NAC had similar effect in 32D cells. GF withdrawal led to Bax translocation to the mitochondria. To further define possible steps along the intrinsic cell death pathway, which are susceptible to regulation by ROS or survival proteins we also analyzed changes in the mitochondrial membrane potential following growth factor removal. The drop in mitochondrial membrane potential after IL-3 removal was suppressed by RAF and NAC treatment.

Conclusion: The data presented here further confirm the essential role of ROS in cell death induction following growth factor withdrawal. They also suggest additional levels of regulation by RAF in the preventing the activation of the intrinsic cell death pathway.

41 TIS7 regulates pICln methylosome complex subunit expression.

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TPA Induced Sequence 7 (TIS7) was characterized by our laboratory as a transcriptional co-repressor [1]. The generation of TIS7 knockout mice showed that TIS7 is involved in muscle differentiation and regeneration [2] as myoblasts of these mice displayed a fusion phenotype. Using MALDI-TOF/TOF in immunoprecipitates of TIS7, we detected all three known components of the methylosome complex [3, 4] protein arginine N-methyltransferase 5 (PRMT5), pICln and methylosome protein 50 (MEP50). The TIS7 complex contained methyltransferase activity *in vitro*. FRET experiments with NIH3T3 cells confirmed the co-localization of the methylosome complex with TIS7 *in vivo*. GST pull-down analyses showed that TIS7 directly interacts with all 3 methylosome subunits.

We have observed a reduction of methylosome subunits expression in TIS7 KO myoblasts on mRNA and protein levels. The next goal is to identify the underlying mechanism.

Here we show that TIS7 regulates ICln transcription, measured by the ICln-luciferase reporter and quantitative real-time PCR assays. Furthermore, we demonstrate that TIS7 regulates ICln expression in a histone deacetylase (HDAC)- dependent manner, since the ICln levels in myoblasts were significantly affected by the Trichostatin A (TSA), a HDACs inhibitor, treatment.

Our data show for the first time that the methylosome subunit ICln affects myoblast fusion. Secondly, we found out that TIS7 regulates methylosome complex subunits expression, and thereby may play a role in a crucial step of cells metabolism, namely the spliceosome assembly.

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42 Structural determinants of $Ca_V1.3$ L-Type Calcium Channel gating

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Background: $Ca_v1.3$ L-type Ca^{2+}-channels are key signal molecules for hearing, cardiac pacemaking and neuronal excitability. We have recently discovered a C-terminal intramolecular protein interaction in $Ca_v1.3$ α_1-subunits as a gating modifier (CTM). This modulatory domain is absent in short C-terminal splice variants. Its presence shifts half maximal activation voltage ($V_{0.5}$) to more positive potentials and inhibits Ca^{2+} dependent inactivation (CDI). This regulation is present in long variants of human and rat cDNA clones from pancreatic islets ($rCa_v1.3_{pan}$) but not in a clone derived from superior cervical ganglion ($rCa_v1.3_{scg}$). $rCa_v1.3_{scg}$ differs from $rCa_v1.3_{pan}$ at three amino acid (aa) positions (S244G, V1104A, A2073V), a poly-M stretch with two additional K, and one alternatively spliced locus (exon 31).

Methods: We systematically compared scg and pan $Ca_V1.3$ α1-subunits by expression in tsA-201 cells and performed whole cell patch clamp analysis.

Results: Two aa (S244, A2073) in $rCa_v1.3_{scg}$ explained most of the functional differences to $rCa_v1.3_{pan}$. Mutation S244G even further enhanced CDI of $rCa_v1.3_{scg}$ and shifted its $V_{0.5}$ to more positive potentials. A2073V (located within the CTM) also shifted $V_{0.5}$ more positive but almost eliminated CDI. The cooperative action in the double-mutant restored gating properties (CDI, $V_{0.5}$) similar to $rCa_v1.3_{pan}$. Their effects are compatible with a recently proposed allosteric CDI mechanism implying CaM-mediated inhibition of the activation gate. Analysis of inactivation kinetics suggest that G244 decreases the open probability (Po) of the inactivated gating mode, whereas V2073 reduces the maximal Po of $rCa_v1.3_{scg}$ as evident from increased gating currents.

Conclusions: G244 and V2073 affect CDI through different molecular mechanisms. Together they stabilize the gating behaviour of long $Ca_V1.3$ splice variants undergoing C-terminal-modulation.

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43 Potassium bromate induces oxidative stress in renal epithelial cells *in vitro* resulting in tight junction rearrangement.

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Background: Potassium bromate (KBrO3) is an oxidising agent that has been widely used in the food industry as a maturing agent for flour. It has shown to be both, a nephrotoxin and a renal carcinogen, in several in vivo and in vitro models. Here we investigated the effects of KBrO3 in the human and rat proximal tubular cell lines, RPTEC/TERT1 and NRK-52E.
Methods: A genome wide transcriptomic screen was carried out from cells treated with a sub-lethal concentration of $KBrO_3$ for 6, 24, and 72 h. Pathway analysis of altered gene expressions was performed and impact on top pathways was further investigated at the protein and functional levels.
Results: The most enriched pathways identified were, "Glutathione metabolism", "Nrf2-mediated oxidative stress" and "Tight junction signaling". The latter pathway was less impacted in NRK-52E cells and further studies revealed an absence of several tight junction proteins in addition to a lack of barrier function development. In RPTEC/TERT1 cells increasing $KBrO_3$ concentrations beyond 1 mM caused a dose dependent GSH depletion and cytotoxicity that was alleviated by co-incubation with 5 mM of the antioxidant N-acetyl cysteine (NAC). Concomitantly, 1 mM $KBrO_3$ exposure caused a decrease in trans-epithelial electrical resistance (TEER) and a decrease in the expression of the tight junction proteins occludin, claudin-2 and claudin-10. Furthermore $KBrO_3$ exposure also caused a cytosolic internalization of these proteins. NAC co-incubation prevented the TEER decrease and also prevented the $KBrO_3$ induced alterations of tight junction proteins.
Conclusion: These results demonstrate that oxidative stress has, in conjunction with the activation of the cytoprotective Nrf2 pathway, a dramatic effect on the expression of tight junction proteins and on barrier function. The further understanding of the cross talk between these two pathways could have major implications for epithelial repair, carcinogenesis and metastasis.

44 ESCRT Mediated Cell Surface Remodeling During Tumorigenesis

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Altered transmembrane protein recycling and degradation may result in loss of cell polarity and abnormal cell migration - two major hallmarks in epithelial tumorigenesis. The five endosomal sorting complexes required for transport (ESCRTs) are involved in the sorting of ubiquitinylated proteins from the cell surface into multivesicular bodies – leading to either their degradation or recycling and have been proposed to function as tumor suppressors.

Yet, the role of ESCRTs in tumorigenesis is still poorly understood.

To address if ESCRTs act as tumor suppressors, inducible lentivirally delivered shRNA constructs are used to knock down selected proteins from each ESCRT-complex in a non-tumorigenic mouse mammary gland epithelial cell line (EpH4). At first, the effects are monitored on a functional level in terms of cell polarity (immunofluorescence), cell layer integrity (transmembrane resistance measurement), cell migration and proliferation potential. Simultaneously, localization and dynamics (confocal and TIRF microscopy) of elected growth factors and adhesion molecules are investigated. In parallel, Ras transformed EpH4 mutants are employed to test if loss of ESCRT function will modify the oncogenic potential of different Ras mutants. Preliminary data from Hela (human cervical carcinoma) and U2OS (human osteosarcoma) cells with an inducible hVps24 knock down construct show a deranged phenotype and increasing cell death respectively.

To unravel the underlying mechanisms of those phenomena, the comparison of the oncogenic potential of ESCRT-mutated EpH4 and EpRas provides a potent tool and will help to clarify if ESCRTs function as tumor suppressors.

45 Quantitative Proteomic Analysis of Multivesicular Body Sorting

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Four protein pathways mediate protein degradation in eukaryotic cells. The endoplasmic reticulum associated protein degradation (ERAD) and the ubiquitin-proteasome system (UPS) pathway are utilizing the proteasome, while autophagy and the MVB pathway target proteins into the lysosme for degradation. Stress response mechanisms for most of these pathways (ERAD -> unfolded protein response, UPS -> heat shock response), are known. However it is not clear how cells react to the loss of the MVB pathway. Since the MVB is, to the best of our knowledge, required for the degradation of most if not all transmembrane-proteins, we are interested in identifying possible stress response pathways. Therefore we initiated two complementary screens to compare wild type cells with ESCRT-Mutants (*vps4∆*) that disrupt the function of the MVB pathway. The first screen compared gene expression levels (RNA - Affymetrix chip) and the second screen measured protein abundances via SILAC (Stable isotope labeling of Amino Acids in cell culture). We have analyzed two biological replicates and identified 3575 proteins and quantified 90% of them (3211 proteins). Of those 254 proteins were significantly regulated. Data integration analysis was performed to correlate SILAC and gene-expression data. Good correlation of mRNA and Protein levels was found for 38 regulated genes. Computational analysis of the significantly up-regulated proteins revealed that the majority of them are involved in amino acid catabolic processes, while the most of the down-regulated proteins are involved in amino acid biosynthetic processes. Free amino acid determination in wt cells and *vps4∆* cells indicate that there are less free amino acids in *vps4∆* cells. Consistently *vps4∆* mutants grow poorly under amino acid limiting conditions. This strongly indicates a defect in the amino acid household, which is subject of further investigations. The combination of gene-expression profiling with quantitative proteomics together with system biological analysis will help us to better understand how cells react to loss of the MVB pathway.

46 Synaptic release and synapse formation in neuronal calcium channel $\alpha_2\delta$ subunit knockdown/knockout models

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Background: Auxiliary $\alpha_2\delta$ subunits are involved in the trafficking and modulation of voltage-gated calcium channels (VGCCs). Furthermore, recent findings suggested a function of $\alpha_2\delta$ subunits in synapse formation. However, whether and how $\alpha_2\delta$ subunits regulate synapse formation and presynaptic transmitter release in differentiated neurons of the central nervous system is largely elusive. Here we analyzed synapse density (immunocytochemistry) and synaptic release (FM dye) by shRNA knockdown and overexpression of $\alpha_2\delta$-1 in highly differentiated cultured mouse hippocampal neurons (DIV 17-24) of wildtype or $\alpha_2\delta$-null background.

Results: Consistent with a potential role of $\alpha_2\delta$-1 in presynaptic function overexpressed pHlourin-tagged $\alpha_2\delta$-1 was highly enriched in the axonal plasma membrane. Surprisingly however, highly efficient $\alpha_2\delta$-1 knockdown did not affect synaptic release rates. In order to exclude functional compensation by other $\alpha_2\delta$ isoforms, we are analyzing cultures from $\alpha_2\delta$-3 knockout mice. Experiments employing $\alpha_2\delta$-1 shRNA knockdown in $\alpha_2\delta$-3 knockout neurons indicate no significant difference in kinetics and amplitude of synaptic release in comparison with $\alpha_2\delta$-3 deficient neurons. In contrast gabapentin, a pharmacologic blocker of $\alpha_2\delta$-1 and -2 significantly reduced the release amplitude. Pre- and postsynaptic specializations were similarly observed in differentiated 21-29 DIV old $\alpha_2\delta$-1 knockdown/ $\alpha_2\delta$-3 knockout neurons; both in efferent (axonal) and in afferent (dendritic) synapses of the transfected neuron.

Conclusion: Together these results suggest that transmitter release in differentiated cultured hippocampal neurons relies on VGCC complexes incorporating the $\alpha_2\delta$-2 subunit. In addition $\alpha_2\delta$-2 might suffice for functional synaptogenesis in this CNS model system. These findings correlate well with the severe neurologic phenotypes observed in $\alpha_2\delta$-2 mutant mice, including seizures, ataxia and abnormal neuronal development.

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47 Investigating the role of RAIDD in Caspase-2-dependent tumor suppression

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The "PIDDosome", is a multi-protein complex containing the proteins PIDD, RAIDD and procaspase-2, which are interacting via homotypic interactions using the death domains (DD) in PIDD and RAIDD, as well as the caspase recruitment domains (CARD) in RAIDD and caspase-2, respectively. This complex is proposed to act as an activation-platform for the cell death-associated protease caspase-2 in response to DNA damage.

In order to investigate the physiological role of the PIDDosome in vivo, *caspase-2*, *pidd* and *raidd* knockout mice have been generated and investigated, revealing a tumor suppressive role for caspase-2, but an oncogenic one for PIDD in a model of c-myc driven lymphomagenesis, while DNA-damage triggered cell death appeared to occur normally in these mice. Consistently, tumorigenesis induced by DNA-damage was not affected by loss of either PIDDosome component (Manzl et al., submitted). Similarly, mice lacking *raidd*$^{-/-}$ did not show an acceleration of disease onset in bulky adduct formation induced sarcoma formation or γ-irradiation induced thymic lymphomagenesis.

Using the *Eμ-myc* mouse model of B-cell lymphoma genesis, we and others showed that *Eμ-myc mice lacking caspase-2* develop malignancies significantly earlier (Ho et al., 2009) while *Eμ-myc/pidd*-deficient mice showed prolonged latency compared to control *Eμ-myc* animals (Manzl et al., submitted). Surprisingly, however, our preliminary data suggests that *Eμ-myc/raidd*$^{-/-}$ mice fail to show any significant difference in onset of B-cell lymphoma genesis. Notably, loss of *raidd,* but not that of *pidd*, favored the development of more mature IgM^{+} B-cell over pre-B-cell tumors. Analysis of tumor cell apoptosis using freshly isolated lymphomas revealed that neither *caspase-2* nor *pidd* or *raidd* deficient *Eμ-myc* primary lymphoma cells show a survival advantage, when treated with different doses of anti-cancer drugs for 24 hours.

Taken together, we could confirm published results that *caspase-2* plays a potent role in limiting oncogene induced tumor progression but this effect does not rely on the adaptor-protein *RAIDD* or its interaction partner PIDD.

48 Conditional Gene Ablation of the MAP Kinase Adapter Protein p14 in Dendritic Cells induces a Myeloid Proliferative Disorder

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Background: Dendritic cells are key players of the immune system and link innate to adaptive immune responses. Their major task is the uptake and processing of pathogens and subsequent presentation of antigens to T cells. These processes strongly depend on endosomal/lysosomal trafficking. Conditional gene disruption of the adapter protein p14 in mice demonstrates that the late endosomal p14/MP1-MEK1 signaling complex is required to control endosomal traffic and tissue homeostasis (Teis et al., J Cell Biol, 2006).

Methods: To address the molecular function of p14 in dendritic cells, we generated a conditional knock out mouse model, which allows the specific deletion of p14 in CD11c expressing cells. The effects were analyzed in tissue (histological methods, FACS, ELISA) and primary cell culture (Western Blot).

Results: The knock out mice were viable but developed a severe pathological phenotype resembling a myeloid proliferative disorder (MPD) at the age of two to three months. The most obvious morphological symptoms included enlarged lymph nodes and splenomegaly. The structural morphology of these organs was disarranged and massive leukocyte infiltrates were observed, which could further be identified as dendritic cells. Additionally, the mice developed infiltrates of monocytes and activated dendritic cells in skin and liver. These infiltrates were also surrounded by single T cells being known as the direct interaction partners of activated dendritic cells. The bone marrow of the CD11c-p14 knock out mice was hyperplastic, accompanied by an increase of hematopoietic stem cells. Furthermore a MPD characteristic shift from the granulocytic towards the monocytic/dendritic cell lineage, an increase in the T helper cell population and a decrease of the erythrocyte progenitors were observed. In the serum of the CD11c-p14 knock out mice at the age of 1 to 6 months, Flt3-ligand, a specific cytokine inducing conventional dendritic cell differentiation, was significantly elevated. Additionally, its receptor Flt3 showed an increased surface localization on splenic dendritic cells. Similar observations were made in p14 depleted keratinocytes where the degradation of the EGF receptor was severely disturbed leading to an accumulation on the plasma membrane (Teis D. et al., 2006, JCB). The accumulation of the receptor on the cell surface and the enhanced availability of its ligand resulted in an increased downstream signaling of Flt3 shown by the phosphorylation of the mTOR target p70 S6 kinase 1. This pathway downstream of the Flt3 receptor is known to be crucial for dendritic cell differentiation (Sathaliyawala T. et al., 2010, Immunity).

Conclusion: Finally we can conclude that p14 deletion in dendritic cells severely affects their tissue homeostasis and leads to a MPD.

49 Protein Adsorption on Modified Solid Surfaces: A kinetic study

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Background: When implants come into contact with the host tissue, the initial event prior to cell attachment is the adsorption of proteins on the surface forming a Biofilm. This rapid adsorption process translates the surface of the foreign body into a kind of biological language. The approaching cells respond to this language, contributing to the ultimate outcomes in implantation, tissue culture and cell culture situations. It is highly probable that the composition of the adsorbed protein layer is a critical determinant of the tissue reaction to such implants. Therefore, a controlled presentation and formation of the Biofilm can stimulate a constructive cell response, favoring wound repair and tissue integration.

The Biofilm formation in the lab is influenced by three groups of parameters. *External factors* are basically temperature, pH, ionic strength, and buffer composition and depend on the design of experiment. For physiological relevant studies, these external parameters are fixed. The second group describes the *protein characteristics*. The complex structure of proteins can be decomposed into individual domains exhibiting specific properties like hydrophilic/hydrophobic, polar/non-polar or charged/uncharged. The individual regions of the protein then may interact differently with the surface and neighboring proteins. The last group of parameters concerns the *surface characteristics* including surface energy, polarity, charge and morphology. All three factors together determine the surfaces wettability. In general, surfaces are classified in either hydrophobic (water repellent) or hydrophilic (water attracting) motifs. A common method for measuring the wettability is the contact angle measurement.

The hypothesis that arises is, if the adsorption of proteins depends on the wettability of the surface. When the wettability can be influenced by surface modification methods, it might be possible to control the composition of the Biofilm in a manner that anchoring proteins are mainly deposited on the surface. Further this may lead to gain influence on the attachment of cells in order to establish a proper linkage between the implant and the host tissue.

The aim of this work is to analyze the protein adsorption process in dependence of wettability and adsorption time. It should be determined if topographical features and UV-irradiation – in combination and alone – can influence the wettability. Further, the adsorption kinetic of proteins should be studied as well.

Methods: *Fabrication of structures*

Polymer discs were produced by injection moulding with defined chequered structures ranging from 5 µm to 350 µm element lenght. The quality of the casting was assessed with scanning electron microscopy (done by Fachhochschule Vorarlberg).

Surface modification

The polymer discs were irradiated with UV-light for defined times. The specimen was placed inside an enclosed Polystyrol box with an UV-source (= 254 nm) on top of the box.

Contact Angle Measurement:

Contact angle was assesed by applying the sessile drop method. Therefore a 2 µl droplet of distilled water was pipetted in the centre of the microstructures. Immediately after that, the droplet was photographed with a USB microscope-camera. The angle between the droplet and the surface (clockwise) was determined with the software ImageJ using the Plug-in “Contact Angle” (Manual Points Procedure).

Protein adsorption measurement:

For protein adsorption study, fetale bovine serum was dissolved in culture media and phosphate buffered saline at the concentrations 1 %, 5 % and 10 %. Sample surfaces were exposed to the protein solutions for defined times (short and long-term). The total amount of adsorbed protein was measured applying the BCA protein assay (Fisher Scientific). For comparison, the kinetic was also assessed on standard polystyrene culture dishes. All values were translated in Albumin equivalent values.

Results: It could be shown, that a chequered surface topography can influence the wettability. With decreasing size of the chequerboard structure, the contact angle decreased too – the surface became hydrophobic compared to the plane surface. Further, also the total surface area increased with decreasing structure size influencing the total amount of adsorbed protein.

The irradiation with UV light modified the polymer surface and increased the wettability dependent on exposure time. The decline of the contact angle can be described mathematically as a polynomial function of degree three.

Shortly after exposure of the surfaces to the protein solution a high amount of protein at the surface was detected. This was followed by a decrease of attached protein reaching a local minimum and a slow rise again. In general, hydrophobic surfaces adsorbed more protein than hydrophilic surfaces. This might be due to the increase in overall surface area (topographical features) or to a lowered energy barrier for repelling hydrogen molecules from the hydrophobic surface.

Conclusion By using topographical features in combination with UV-irradiation, it is possible to gain control over the wettability and further to influence the protein adsorption. A simple structure like chequerboard features can be realised without high effort on implant surfaces. Also other structural features can be evaluated for their application as wettability controlling factor. The irradiation with UV light at a wavelength of 254 nm does not only increase the wettability but also sterilizes the surface.

50 Microvillus Inclusion Disease Is A Disorder Of Disrupted Epithelial Cell Polarity

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Objectives and Study:Microvillus Inclusion Disease (MVID) is a congenital enteropathy characterized by a loss of microvilli, the appearance of microvillus inclusions and the cytoplasmic accumulation of Periodic Acid – Schiff (PAS)- positive vesicles in enterocytes. Our recent studies have identified mutations in MYO5B, encoding the unconventional Myosin Vb motor protein, to be causually involved in the pathogenesis of MVID. MyosinVb is implicated in maintaining cell surface polarity in epithelial cells. The aim of this study was to explore the impact on RNAi mediated MyosinVb depletion on the polarized organization of human intestinal CaCo2 Cells, as a model of polarized intestinal epithelium in vitro.
Methods:Myosin Vb knock down was performed in polarized, brush border forming CaCo2 Cells. For polarization, CaCo2 Cells grown to confluency on a Costar Transwell Filter System for 10 days. For Myosin Vb knock down studies, CaCo2 Cells were stably transfected with 2 different lentiviral shRNA vectors for designing an intestinal epithelial cell line with an inducible Myosin Vb knock down. The polarized organization of MyosinVb depleted CaCo2 Cells was determined by Imunnofluorescence Microscopy, Scanning Electron Microscopy and Western Blotting Analysis.
Results: Myosin Vb depleted CaCo2 Cells displayed several features of impaired cell polarity including a loss of microvilli, formation of microvillus inclusions, disorganization of the actin cytoskeleton, a reduction of the transepithelial resistance, loss of adherent junctions and mislocalization of the basolateral transporters GLUT1 and the Na/K ATPase and CD36, a transporter for long chain fatty acid uptake, respectively. Finally, disruption of intracellular trafficking was shown by cytoplasmic mislocalization of effector proteins such the small GTPases Rab11 and Rab8.
Conclusion: Our findings point to a critical role of Myosin Vb in the polarized organization of human intestinal cells thereby defining MVID as a disorder of disrupted epithelial cell polarity.
Acknowledgement: Supported by the Austrian Research Fund and the MCBO program

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51 EHEC-Derived Shiga Toxin 2 Affects Human Plasmatic Coagulation System but not Platelets

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Background: Shiga toxin 2 (Stx2) producing enterohemorrhagic *Escherichia coli* (EHEC) is the most important cause for typical hemolytic uremic syndrome (HUS). In HUS patients renal injury and thrombotic disorders are observed. It has been postulated that Stx is able to activate platelets in a direct way. However, there are also hints that the plasmatic coagulation system is affected in HUS. In this study we investigated the effect of Stx2 on platelets and on antithrombin (AT) as a strong inhibitor of the plasmatic coagulation cascade.
Methods: Aggregometry was used to show the aggregation inducing capacity of Stx2. In flow cytometry, activation of platelets was shown measuring the expression of CD62P (P-selectin) and CD63. Concerning AT, ELISA was used to evaluate whether it binds to Stx2. To elucidate the consequences of this binding, a functional assay based on the automated BCS® XP coagulation system (Siemens) was performed to ascertain the inhibiting functions of AT directed against activated coagulation factor II (CF IIa, Thrombin) and X (CF Xa, Stuart-Prower-factor).
Results: Neither in aggregation, nor in the expression of the investigated activation markers CD62P and CD63, a Stx2-induced effect on platelets could be observed. However, ELISA revealed a strong binding of AT to Stx2. In the functional tests a significant reduction of the anti-factor Xa function of AT was observed at Stx2 concentrations higher than 1.6 ng/ml. No influence of Stx2 on anti-FIIa function of AT was detected.
Conclusion: It appears that platelets are not directly activated by Stx2. However, in this study we show binding of AT to Stx2 which may result in reduced function of AT directed against CF Xa. We hypothesize, that HUS-associated thrombotic disorders are not only caused by platelet adhesion triggered by Stx2-derived endothelial damage, but may also be mediated through direct influence of Stx2 via the plasmatic branch of the coagulation system.

52 Targeting Viral Antigens to CD11c on Dendritic Cells Induces Retrovirus-Specific T cell Responses

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Background: Dendritic cells (DC) represent the most potent antigen presenting cells to induce efficient cytotoxic T lymphocyte (CTL) response against viral infections. Delivering antigens (Ag) to receptors on DCs is intensively studied as promising tool to induce antitumor and antiviral immune response by DCs.
Methods: Here we investigated the potential of CD11c-specific single-chain fragments (scFv) fused to immuno-dominant peptide of Friend virus for the induction of virus-specific T cell response by DCs.
Results: CD11c-specific scFv selectively targeted viral antigens to DCs and thereby significantly improved the activation of virus-specific T cells *in vitro*. DCs loaded with viral Ag targeted to CD11c provided improved rejection of FV-derived tumors and more importantly efficiently primed virus-specific CTL response *in vivo*.
Conclusions: Since the induction of strong virus-specific T cell responses is critical in viral infections, CD11c targeted protein vaccines triggering cellular immunity might provide alternatives beside other vaccination strategies.

53 Screening of Differentially Expressed Genes of *Aspergillus terreus* in Response to Amphotericin B

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Background: Infections with *Aspergillus* sp. have emerged worldwide among patients undergoing cancer chemotherapy, hematopoietic stem-cell transplantation, or solid organ transplantation. Among the different species *A. terreus* takes an exceptional position: most clinical isolates exhibit primary resistance to amphotericin B (amB) treatment, a broad-spectrum fungicidal agent that has been widely used against life threatening fungal infections. *A. terreus* is a common cause of invasive aspergillosis (IA) in some geographically unrelated institutions, such as the University of Texas M. D. Anderson Cancer Center in Houston, Texas, and the University Hospital of Innsbruck, Austria.

Methods: The objective of this study is to identify genes, and further on proteins and/or pathways that might account for amB resistance of *A. terreus*. To profile the genome-wide expression response, microarray analysis was carried out comparing expression levels of an amB resistant *A. terreus* isolate (ATR) to those of an amB susceptible *A. terreus* (ATS) in response to sublethal amB concentrations in a time dependent manner.

Results: In ATR a total of 256 genes ($\log_2$ ratio >1) were up-regulated, 28 genes were down-regulated, whereas in ATS only 18 genes were up-regulated. None of the up-regulated genes of ATS was down-regulated in ATR.

Conclusion: These data demonstrate that resistance to amB requires many genes to be differentially expressed and various pathways might be involved. Further investigations are in process to study the impact of the differentially expressed genes on amB resistance.

54 Secretable antiviral entry inhibitory (SAVE) peptides for gene therapy of HIV infection

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Background: C peptides (e.g. T20, C46) are highly efficient inhibitors of HIV entry. Secreted from gene-modified cells, C peptides are expected to mediate a bystander protective effect on neighboring non-modified cells and suppress virus replication even if only a small fraction of cells is genetically modified.

Methods: Short peptides, such as the C peptides, are only inefficiently translated and exported by the cellular secretory machinery. To circumvent these limitations we expressed therapeutic C peptides as concatemers, which were subsequently processed into monomeric peptides by protease cleavage within the secretory pathway.

Results: Transfection or transduction of cell lines with retroviral vectors resulted in high-level expression and secretion of SAVE C peptides, which exerted a high antiviral activity in single-round infection assays with replication incompetent lentiviral particles pseudotyped with a variety of different HIV envelope glycoproteins. In mixed cell cultures SAVE peptides secreted from transduced cells produced a bystander effect and suppressed HIV-1 infection of non-modified cells.

Conclusion: The in vivo secretion of therapeutic C peptides from gene-modified T or B cells holds great promise as the cells would be expected to home to lymphatic tissues, which are the major sites of HIV replication. Secretion of the antiviral gene product in the lymphatic tissues is likely to lead to high and stable local concentrations and confer a substantial antiviral effect.

55 Binding of Shiga Toxin to Factor H–Related Protein 1

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Background: Typical hemolytic uremic syndrome (HUS), an acute renal disease, is mainly caused by infections with enterohemorrhagic *E.coli* (EHEC) strains. Shiga toxin 2 (Stx2) is a major virulence of EHEC. Recently, an involvement of Stx2 in complement activation and its binding to complement regulatory protein factor H has been described. The aim of our study was to investigate the binding of Stx2 to another member of the FH family, factor H-related protein 1 (FHR1).

Methods: The experiments were performed by ELISA. Stx2 was immobilized onto microtiter plates. After blocking, FH or FHR1 were introduced, and the bound proteins were detected with polyclonal factor H antiserum and a secondary anti-sheep IgG conjugated with alkaline phosphatase. The reaction was developed with the chromogen substrate 4-nitrophenylphosphate, and absorbance was measured at dual wavelengths of 415 and 490 nm.

Results: Stx2 does not only bind to FH, but also to FHR1 in a dose dependent manner and, in addition, FHR1 binding appears to be more pronounced than FH binding. FH binding is dose-dependently decreased in the presence of increasing concentrations of FHR1. Stx2 binds to the short consensus repeats (SCRs) 3-5 of FHR1, resembling SCRs 18-20 of FH, while it does not bind to SCRs 1-2 resembling SCRs 6-7 of FH. Two allotypes of FHR1 (FHR1*A and FHR1*B) also bind to Stx2 in dose dependent manner and it appears that Stx2 binds better to FHR1*A, which is found less frequently in atypical HUS, than to FHR1*B. In addition, it is the Stx beta subunit which binds to both FH and FHR1.

Conclusion: In addition to FH, Stx2 can also bind to FHR1 in which SCRs 3-5 represent the binding site. Both proteins compete for binding to Stx2. Stx2 binds to FHR1*A better than FHR1*B, suggesting that this may give some protection against typical HUS. In addition, the Stx beta subunit which is responsible for binding to receptors on host cells, is the region of the toxin that binds to FH and FHR1.

56 Determining the Potential of Vesicular Stomatitis Virus Pseudotyped with the Glycoprotein of the Lymphocytic Choriomeningitis Virus as Vaccine Vector

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Background: Vesicular stomatitis virus (VSV) as a vaccine vector has already been shown to induce potent immune responses in several studies using viral or tumor antigens. The biggest limitation of VSV however, is its neurotoxicity which prevents application in humans. The neurotoxicity is mainly mediated by the glycoprotein (G). Our group has recently shown that a VSV pseudotyped with the glycoprotein (GP) of the lymphocytic choriomeningitis virus (LCMV) is not neurotoxic. Moreover, as neutralizing antibodies against GP are hardly induced (in contrast to the VSV glycoprotein), boosting with the same vector should be possible for the pseudotyped VSV (VSV-GP). **Methods:** The aim of this work is to evaluate the potential of VSV-GP as a vaccine vector. For this purpose we use Ovalbumin (OVA) as model antigen, for which the immunogenic epitopes are characterized and well established animal models and tools to evaluate immunogenicity are available. Immunogenicity of GP-pseudotyped and wildtype VSV containing OVA (VSV-GP_OVA and VSV_OVA) is analyzed *in vitro* and *in vivo* in mouse models. **Results:** We could show that both vectors (VSV_OVA and VSV-GP_OVA) infected murine bone marrow derived dentritic cells (bmDCs) *in vitro*. These infected bmDCs were able to activate OVA specific $CD8^+$ T cells as well as $CD4^+$ T cells *in vitro*. T cell activation was comparable for both viruses. First immunization experiments in mice revealed, that VSV-GP was able to activate OVA specific cytotoxic T cells (CTLs) after one single application. The response for VSV-GP_OVA was even stronger compared to VSV_OVA. **Conclusion:** Taken together, we could show that the non-neurotoxic VSV-GP is able to mount a strong T cell response both *in vitro* and *in vivo*. These preliminary data demonstrate that VSV-GP has the prerequisite for a potent vaccine vector.

57 Evaluation and Optimisation of Radiolabelled Liposomes targeting Tumour-induced Angiogenesis

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Background: Radiolabelled liposomes are promising vehicles, which are used in clinical applications such as diagnostic imaging and radionuclide therapy. The aim of this study was to evaluate and optimise radiolabelled PEGylated liposomes derivatised with cyclic RGD-peptides (RLP). These RGD-peptides bind to $\alpha_v\beta_3$-integrin receptors, over-expressed during tumour-induced angiogenesis, and have already been used for drug delivery studies, tumour therapy, and vascular targeting.

Methods: RLP were synthesised according to the lipid film hydration method. Different phospholipids, PEGylated building blocks, DTPA/NOTA-derivatised lipids for radiolabelling with In-111 or Ga-68, and a lipid-based RGD building block were used. Relative amounts of RGD- and PEG-building blocks were varied and additionally a fluorescent label was added. To analyse *in vitro* binding affinity plates coated with $\alpha_v\beta_3$-integrin receptors were used and incubated with varying concentrations of radiolabelled RLP. Biodistribution and small animal SPECT imaging were performed in nude mice bearing $\alpha_v\beta_3$-positive M21 and $\alpha_v\beta_3$-negative M21L tumour xenografts.

Results: Radiochemical purity of RLP was >95 %. *In vitro* binding studies showed a) good binding to isolated receptors, b) differences between high and low RGD-loading, and c) a strong influence of PEGylation. Binding could be improved with higher RGD-loading, whereas binding was reduced with higher PEGylation. Fluorescence studies supported these results. Biodistribution data revealed a good stealth effect and long circulating ability for PEGylated RLP. Dependence of RGD-loading could be confirmed *in vivo* with a higher tumour uptake of RLP with higher RGD-loading. Small animal SPECT imaging displayed only moderate uptake in the tumour and high uptake in liver and spleen.

Conclusion: Here we describe the optimisation of radiolabelled liposomes carrying a RGD building block to target angiogenesis. Even though good binding to $\alpha_v\beta_3$-integrin receptors *in vitro* was found, an optimised balance between PEGylation and RGD-loading is required to achieve optimal targeting *in vivo*. These data form the basis for future optimisation steps to investigate multifunctional approaches.

58 MRI Molecular Imaging with Targeted Albumin-based Nanoparticles: Conceptual Design Strategies to create the Magic Bullet

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Background: Paul Ehrlich defined „Magic Bullets" as targeted vehicles carrying a drug [1], and they are realisable as Richard Feynman's nanomachines [2]. Application of such constructs in Nanomedicine promises to improve drug delivery and reduce dosages and side-effects ("personalized medicine"). Two major challenges must be overcome: 1. identification of suitable biomarkers; 2. construct nanoparticles homing to biomarker molecules behind intact tissue barriers.

Methods: We developed and characterized albumin-based nanoparticles bearing gadolinium. The lectin LEA was attached to the particles to target oligolactosamines. Coordinated with MRI, chemical and immunohistochemical analyses tracked components of the nanoparticles for 15 minutes to 6 weeks, obtaining large runs of quantitative data.

Results: Nanoparticles were ~30 nm diameter, were pure, and had good imaging properties (relaxivities $\sim 1 \cdot 10^7$ 1/Ms). They were stable in various *in vitro* testings but not in SDS-gel-electrophoresis. They haemagglutinated red blood cells; after intravenous injection into living rats they gave high-resolution MR Molecular Imaging of the vascular wall lasting >2 hours.

Conclusions: We obtained MR Molecular Imaging by use of nanoparticles; they require an addition type of targeting group to access subendothelial compartments, for Molecular Targeting of disease sites behind intact tissue barriers. Multiple targeting is new in Nanomedicine [3], using it we can now design nanoparticles to target drugs and contrast agents to disease lesions behind blood-tissue barriers: multiply-targeted nanoparticles are Ehrlich's "magic bullets".

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59 MtDNA Lineages D1g and D1h: New Insights into the Pioneer Peopling of South America's Southern Cone

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Background:

It is now widely agreed that the Native American founders originated from a Beringian source population ~15-18 thousand years ago and rapidly populated all of the New World, probably mainly following the Pacific coastal route. However, details about the migration into the Americas and the routes pursued on the continent still remain unresolved, despite numerous genetic, archaeological and linguistic investigations.

Methods:

To examine the pioneering peopling phase of the South American continent, we screened literature and mtDNA databases and identified two novel mitochondrial DNA (mtDNA) clades, here named D1g and D1h, within the pan-American haplogroup D1. They both show overall rare occurrences, but local high frequencies, and are essentially restricted to populations from the Southern Cone of South America (Chile and Argentina). We selected and completely sequenced 43 D1g and D1h mtDNA genomes applying highest quality standards.

Results and Conclusion:

Molecular and phylogeographic analyses revealed extensive variation within each of the two clades and possibly distinct dispersal patterns. Their age estimates agree with the dating of the earliest archaeological sites in South America and indicate that the Paleo-Indian spread along the entire longitude of the American double continent might have taken even less than 2,000 years. This study confirms that major sampling and sequencing efforts are mandatory for uncovering all of the most basal variation in the Native American mtDNA haplogroups and for clarification of Paleo-Indian migrations, by targeting, if possible, both the general mixed population of national states and autochthonous Native American groups, especially in South America.

60 Mitochondrial RNaseP Function in HSD10 Disease

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Loss of function of the mitochondrial enzyme 17β-hydroxysteroid dehydrogenase type 10 (HSD10) causes 2-methyl-3-hydroxybutyryl-CoA (MHBD) dehydrogenase deficiency, an organic aciduria with a neurodegenerative course and evidence of mitochondrial dysfunction. Seven mutations in the corresponding *HSD17B10* gene have been identified so far. Interestingly, disease severity is not correlated to the dehydrogenase activity. These findings suggest a novel function of the HSD10 protein.
Recently HSD10 was found to be part of the ribonuclease P (RNase P) protein complex in mitochondria. RNase P is an endonuclease consisting of three proteins (MRPP1, HSD10, MRPP3) and is responsible for the 5`-maturation of precursor mitochondrial transfer RNAs (pre(mt)-tRNAs). It was shown that all three proteins are required for a functional enzymatic activity of RNase P cleaving different pre(mt)-tRNAs. Here we analyzed whether HSD10 mutations found in patients alter mtRNase P function. Expression of the three mtRNase P subunits in protein extracts from patient fibroblasts and HSD10 knock-down HEK cells were determined by western blot analysis. Both HSD10 and MRPP1 showed markedly reduced expression in patient fibroblasts and knock-down cells compared to control cells. Expression of MRPP3 was not affected. Quantitative RT-PCR analysis of different pre(mt)-tRNAs sequences in HSD10 knock-down cells caused accumulation of tRNA precursors. MRPP1 mRNA was unchanged compared to controls. Surprisingly, pre(mt)-tRNA levels in patients` fibroblasts did not differ from control cells. Taken together our results show that HSD10 mutations reduce RNase P levels in fibroblasts but did not change mitochondrial tRNA processing. Therefore, we conclude that additional HSD10 modulated pathways are affected in MHBD deficiency.

61 Breakpoint Characterization in a Patient with a Complex Rearrangement of Chromosome 7 Including a dup(7)(q22.1q32.2) and an inv(7)(q31.31q31.33)

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For the understanding of the impact of small chromosomal rearrangements, the knowledge of the exact breakpoint-position is important, but breakpoint characterization on the base pair level is rare to find in the literature. However, by high resolution CGH- or SNP-arrays it is possible to localize the breakpoint region to a size that can be detected by simple PCR techniques. Here, we report on the breakpoint characterization of a *de novo* 46,XY,der(7)(pter→q31.33::q31.31→q22.1::q32.2→q31.33::q31.33→q32.2::q32.2→qter) karyotype in a 12-year-old boy with multiple congenital anomalies and mental retardation.

The size and the breakpoints of the duplication were narrowed with the Illumina® Infinium HD HumanOmni1-Quad v1.0 BeadChip, which covers more than 1 million SNPs, between rs17720576 (98.616.657 bp) and rs10275844 (98.621.315 bp) and rs10954272 (130.296.528 bp) and rs6467310 (130.313.744 bp), respectively. So, the size of the duplication as a whole is about 31,7 Mb. Breakpoint-spanning long-range PCR revealed a PCR product of approximately 18 kb. Sequencing of this product is in progress and should identify the duplication breakpoints on the base pair level.

The distal breakpoint of the inversion which is about 25 Mb in size was narrowed by fluorescence in situ hybridization (FISH) with the breakpoint-spanning probe RP11-111J7 (BlueGnome®, Cambridge, UK) between 117.631.652 bp and 117.790.729 bp. Fine-mapping of the proximal breakpoint of the inversion by FISH is in progress.

In conclusion, combination of FISH and high-resolution CGH- or SNP-arrays allows narrowing the breakpoints in complex chromosome rearrangements more easily than previously applied techniques. This could be important for a better genotype-phenotype correlation.

62 Concurrent Quantitative- Real-time PCR Transcription Study of Mutant and Wildtype Alleles in Smith-Lemli-Opitz-Syndrome

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The Smith-Lemli-Opitz-Syndrome (SLOS) is an autosomal recessive metabolic malformation disorder. It is caused by mutations in the *DHCR7* gene encoding the Δ7-sterol reductase. About 90% of mutations reported are predicted to cause amino acid changes, however the impact on splicing or RNA stability is unknown. Aim of the study was to establish a technique to analyse the transcription efficiency depending on mutations in the gene, and to study the effect on transcription of SLOS causing mutations. Hydrolysis probe based qPCR technique is used to compare the transcription efficiency of the mutated allele in comparison to the wt allele within one cell. By the applied method one may calculate directly the input amount of the two transcripts.
Transcriptional activity of the *DHCR7* gene was assessed by quantitative real-time PCR in heterozygous fibroblast cell lines from parents of SLOS patients. The experiments have shown for different *DHCR7* mutations (c.964-1G>C, p.Thr93Met, p.Trp182Cys, p.Glu224Lys and p.Gly410Ser) that they are transcribed in the same amount as the wild type alleles. The splice site mutation c.964-1G>C leads to a premature stop, but does not trigger nonsense mediated decay (NMD) due to localization of the mutation in the last intron. For the null mutation p.Trp151X NMD has already been reported. We could confirm this former result and detect the altered allele by use of puromycin.
Our quantitative PCR technique allows the analysis of transcription levels of two different alleles in the same cell line independently of transcription level of the investigated gene. Transcript stability of the alleles carrying c.964-1G>C, p.Thr93Met, p.Trp182Cys, p.Glu224Lys and p.Gly410Ser are not impaired compared to the normal allele. With the more sophisticated PCR technique the impact of mutations on the RNA level can be observed easily. This technique allows also declaring about the RNA stability in the cell.

63 Characterization of mtDNA SNP Typing Using Quantitative Real-time PCR for Forensic Purposes

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Background

The analysis of mitochondrial DNA (mtDNA) is highly useful in forensic casework, particularly when analyzing challenging samples. Yet, mtDNA testing does not provide definitive identification of individuals as all members of a matriline are expected to match each other due to maternal inheritance of the mtGenome. Hence, a low power of discrimination is obtained when common mitochondrial types are present. Sanger sequencing of the non-coding control region (CR) of the mtGenome is the gold standard in forensic mtDNA testing. Nevertheless, to gain additional information, CR sequencing can be supplemented by assays selectively addressing single nucleotide polymorphisms (SNPs) in mtDNA testing.

Methods

An Austrian population sample (405 individuals with known CR sequences) was investigated by real-time PCR using TaqMan probes interrogating the mtDNA SNP 16519T/C.

Results

The TaqMan approach facilitated unambiguous allele calling for the mtSNP 16519. For both alleles, the analytical window covered ≥5 orders of magnitude (1,000,000-10 dsDNA targets). The apparent single cycle exponential phase PCR efficiencies were close to 100%. Defined mixtures of cloned 16519T and C amplicons were reliably detected and quantified down to the 5% level for either variant (sensitivity: 100-200 plasmids). The estimated mixture ratios for heteroplasmic and homoplasmic samples were consistent with the results obtained by clone typing (~300 clones/sample).

Conclusions

The wide analytical window and the short amplicon size, facilitate the analysis of a broad spectrum of samples differing in DNA quantity and quality with a single protocol.

The quantitative information gained aids the formulation of objectively-based criteria for distinguishing authentic signal and contamination.

The homogeneous assay format avoids potential cross-contamination and sample mix-up and makes it automation friendly.

Offline amplification with post-PCR signal read-out facilitates very high sample throughput.

64 Novel substrate proteins of protein arginine methyltransferases for Aspergillus nidulans

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Background. In the filamentous fungus Aspergillus nidulans three distinct protein arginine methyltransferases (PRMTs) are present which all possess in vitro and in vivo methyltransferase activity. One of these enzymes, termed RmtB, has an exceptional position because it displays both enzymatic and structural properties in comparison to other known PRMTs. Thus, our long term objective is to clarify the functional role of these fungal PRMTs. For this purpose we want to separate PRMTs protein complexes, to identify interacting proteins and to isolate novel substrate proteins, to finally get more information on the implication of these enzymes in fungal specific pathways.
Methods. Recently, we have generated mutants of the corresponding PRMT genes by targeted gene replacement. Protein extracts of mutant mycelia were separated by ion exchange chromatography (IEX) and resulting fractions were used for the in vitro labeling of proteins with purified RmtB (IEX, gelfiltration). Labeled substrate proteins were further separated and analyzed by 2D gel electrophoresis and fluorography, respectively, and will be subsequently identified by mass spectrometry.
Results. In an initial screen, we could identify several selective and non-selective proteins as putative targets of Aspergillus PRMTs. The subsequent analysis of these protein fractions by 2D gel electrophoresis allowed the high resolution separation for the identification by mass spectrometry.
Conclusion. Our results have confirmed our previous findings that demonstrated significant in vitro methylation potential of endogenous PRMTs and the presence of a variety of yet unknown substrate proteins in Aspergillus nidulans. Importantly, our in vitro methylation assays revealed the presence of selective and non-selective substrate proteins in Aspergillus protein extracts depending on the initial methylation status of these proteins in vivo.

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65 Revealing the elusive Molecular Biology of the vault RNA

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In the recent past, the importance of the surprisingly diverse class of non-protein-coding RNA molecules (ncRNAs) has been widely recognized. The key feature of all ncRNAs is that they are not translated into proteins but rather exert their functions at the RNA level. They play key roles in a variety of fundamental processes in all three domains of life. Their functions include DNA replication and chromosome maintenance, regulation of transcription and translation, RNA processing, protein synthesis and stability of mRNAs, and even regulation of stability and translocation of proteins. Many ncRNAs have been discovered fortuitously, suggesting they merely represent the tip of the iceberg [1].In a recent genomic ncRNA screen we have identified the vault-associated RNAs to be significantly up-regulated in human B cells upon Epstein-Barr virus (EBV) infection [2]. Vault RNAs serve as integral parts of the so-called vault complex, a large hollow barrel-shaped ribonucleo-protein complex with a size of 13 MDa [3]. Very little is known about the function of this ncRNA class, mainly because the vault complex has been overlooked by cell biologists for many years.
As the up-regulation of vault RNA seems indeed to be causally linked to EBV [4], we were interested which function the vault RNAs might possess during virus propagation. We could show that the presence of the vault RNA leads to a better viral establishment and protects cells from undergoing apoptosis. Furthermore the hypothesis could be supported that these effects appear to be a function of the vtRNA and not of the vault particle. Cumulatively, the further planned experimental strategy will eventually reveal the so far enigmatic molecular biology of this interesting ncRNA species.

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66 Identification of ATP-dependent mechanisms of centromeric chromatin assembly in Drosophila

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The centromere is a part of the chromosome which is crucialy important for segregation of chromosomes during cell division and cohesion of sister chromatids. The centromere-specific histone CENP-A, which is known as CID in Drosophila, plays an important role in determining centromeric functions. CID is a variant version of histone H3 and is incorporated into centromeric nucleosomes in a replication-independent manner during mitosis in *Drosophila*. Chromatin assembly is usually carried out by a concerted action of histone chaperones and ATP-dependent remodeling enzymes, which belong to the SNF2 superfamily of ATPases. So far, however, no SNF2-like factor has been identified to be involved in CID incorporation in the fly.

The goal of this project is to find SNF2-type remodelers that contribute to the incorporation of CID into centromeric chromatin. To this end, we have chosen 12 members of the SNF2 family for the analysis of their effects upon RNAi-mediated downregulation in S2 cells. Knock-down of individual factors and their combinations, so far revealed effects on cell proliferation and viability as well as mitotic arrest phenotypes. However, none of the studied factors appeared to affect CID deposition. Thus, in a complementary approach we are know seeking to identify potential CID loading factors by the purification of CID-containing protein complexes from different cellular compartments.

This project is founded by Marie-Curie ITN "Nucleosome 4D" (http://www.nucleosome4d.net/)

67 HosA, a major regulator of secondary metabolism in Aspergillus nidulans

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Background: Filamentous fungi produce a variety of so-called secondary metabolites (SM), low molecular weight compounds with a multitude of biological functions. Many of them are used as pharmaceuticals such as antibiotics, but also most potent toxins and carcinogens are natural products of fungi.

Genetic studies augmented by analysis of whole genome sequences revealed, that most fungal SM biosynthetic genes are found in compact clusters functioning as individual genetic loci. Notably, many of these clusters are silent under growth conditions routinely used in laboratories thus biological features of the encoded metabolites are uncovered yet.

Histone deacetylases (HDACs) catalyze the removal of acetyl groups from distinct lysine residues of the N-terminal tails of the core-histones H2A, H2B, H3, and H4 and it is increasingly evident that these enzymes are also critical factors for the regulation of fungal secondary metabolites.

Methods: In this study we have deleted *hosA,* a gene encoding a class 1 type HDAC of *A. nidulans*. Generated strains were grown under various conditions and were phenotypically analyzed. Moreover, production of SMs was determined by transcription analysis and penicillin bioassay, respectively.

Results: We demonstrate that deletion of *A. nidulans hosA* causes severe growth deficiencies and considerably impacts the transcription of secondary metabolite gene clusters, which is either increased or repressed, dependent on the cluster analyzed or the growth conditions used.

Conclusion: HosA turns out to be a major regulator of SM production in filamentous fungi. Regulation might either occur directly via alteration of the chromatin structure, or indirect by the interaction with respective SM-regulators. In any case, the transcriptional regulation of SMs by HosA appears to be precise and highly specific, since neither actin nor flanking genes of the SM clusters are affected in *hosA* deletion strains.

68 Application of a Sheathless Capillary Electrophoresis-Mass Spectrometry (CE-MS) Platform for Peptide Analysis - Comparison to nanoLC-MS

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Background: In this study we have evaluated the suitability of a sheathless capillary electrophoresis-mass spectrometry (CE-MS) interface with a porous tip as nanospray emitter for the use in peptide analysis. After having optimized the separation conditions and instrument parameters, we investigated the application of the CE-MS method for the characterization of complex mixtures consisting of distinctly acetylated, phosphorylated, methylated and deamidated histone proteins as well as microsequence variants differing only slightly in mass and charge.

Methods: A positively charged capillary coating and 0.1% formic acid as background electrolyte were used for CE separations upstream from mass spectrometry characterization. The sheathless CE-MS method developed was compared with nanoLC-MS for the analysis of ArgC-digested rat linker histones.

Results: As shown in this investigation, the spray performance, dependent on the distance between emitter tip and MS orifice as well as on the voltage applied, the injection conditions, and the strength of the EOF have significant influence on the efficiency of the system. Under optimized conditions the system generated efficient and stable ESI resulting in high signal-to-noise ratios and sensitivity. In order to compare the effectiveness of the CE-MS method developed with a nano-LC-MS method, Arg-C digested rat testis linker histones were analyzed. With comparable amounts of sample applied to both systems, the number of identified peptides increased by more than 60% when using CE-MS, and the analysis time was significantly reduced. We found that low molecular mass peptides (below 1400 Da) were preferentially identified by CE-ESI-MS, since this group of peptides poorly interacted with the reversed phase material in the nanoLC system.

Innovative Aspects:

Based on the results obtained, CE-MS can be considered as a complementary technique to conventional LC-MS and as an alternative approach for modern protein/peptide analysis.

69 To the origin of translational control: Are there small ncRNAs interacting with the archaeal ribosome?

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The functions of ribosomes in translation are complex and involve different types of activities critical for decoding the genetic code, linkage of amino acids via amide bonds to form polypeptide chains, as well as the release and proper targeting of the synthesized protein. During the elongation cycle the ribosome has to interact dynamically with various RNA (e.g. mRNA), RNP (e.g. aminoacyl-tRNA-EF-Tu) and protein ligands (e.g. EF-G, RF). Since ribosomes are so fundamental to life, understanding how they work and how they are regulated during protein biosynthesis is at the heart of molecular understanding of biology.

Non-protein-conding RNAs (ncRNAs) showed in the last years to be crucial in regulatory networks (e.g. chromosome remodelling; RNA polymerase activity; mRNA turnover; etc). However all of the recently discovered ncRNAs involved in translation regulation target the mRNA rather than the ribosome. This is unexpected given the central position the ribosome plays during gene expression and the assumption that the primordial translation system most likely received direct regulatory input from small molecules including ncRNA cofactors.

The main goal of this project is to identify potential novel ncRNAs that directly bind and possibly regulate the ribosome during protein biosynthesis. To address this question our group has already started genomic screens for novel regulatory ncRNAs that associate with Saccharomyces cerevisiae ribosomes under specific environmental conditions.

Recently we have expanded our genomic screens to two novel model organisms: the extremely halophilic archaea Haloferax volcanii and the basal flatworm Macrostomum lignano. These organisms can hopefully give us a better insight into the potential primordial regulation of translation. In order to select for ribosome-associated ncRNAs, we apply various stress conditions, isolate the ncRNA-ribosome-complexes and subsequently the small ncRNA candidates that co-purify with the translating ribosomes are used for cDNA library construction. The library has then been subjected to deep-sequencing analyses and potential ncRNAs are tested for their presence and function during the ribosomal elongation cycle.

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70 The interplay of vacuolar and siderophore-mediated iron storage in the opportunistic fungal pathogen *Aspergillus fumigatus*

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Background: Iron is an essential element for all eukaryotes but its excess is deleterious. Iron homeostasis results from tight regulation of iron acquisition and iron storage to ensure sufficient iron supply and to prevent iron toxicity. *A. fumigatus* produces the extracellular siderophores (low-molecular mass iron chelators) triacetylfusarinine C (TAFC) and fusarinine C (FSC) for iron uptake and the intracellular siderophores ferricrocin (FC) and hydroxyferricrocin for distribution and storage of iron. Siderophore biosynthesis is important for adaptation to iron starvation and therefore crucial for virulence. Intracellular iron excess has been shown to increase the content of FC-chelated iron and the expression of AFUA_4g12530, termed CccA, which shows similarity to the vacuolar iron importer Ccc1 of *S. cerevisiae*. These data indicate a role of both the vacuole and FC in iron detoxification.

Methods: The function of *A. fumigatus* CccA and the interplay of vacuolar and FC-mediated iron storage was characterized by analyzing the consequences of deletion or overexpression of *cccA* in various genetic backgrounds.

Results: Green fluorescence protein-tagging confirmed localization of CccA in the vacuolar membrane. During high iron conditions genetic inactivation of CccA impaired growth in various genetic backgrounds, in particular in combination with derepressed iron uptake due to deficiency in the iron regulator SreA. In contrast, overproduction of CccA increased iron resistance. Inactivation of FC biosynthesis did not affect iron resistance. Lack of FC, CccA and in particular both, increased the cellular content of iron chelated by FSC/TAFC breakdown products. These data indicate that the transfer of iron precedes recycling of FSC/TAFC degradation products, which might represent another iron detoxifying mechanism.

Conclusions: Taken together, these data indicate that vacuolar rather than FC-mediated iron storage is the major iron detoxifying mechanism of *A. fumigatus.*

71 Identification of Novel Compounds that Modulate FOXO3 Activity

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Background: FOXO3 belongs to the forkhead box O family of transcription factors and is known to be dysregulated in many malignancies, including cancer. Originally, it has been considered as tumor suppressor, since FOXO3 activation leads to the expression of pro-apoptotic target genes like Bim, Noxa, Puma and TRAIL. However, there is rising evidence that dysregulated FOXO3 activity can also promote tumor progression via upregulation of target genes involved in oxidative stress response, drug resistance and metastasis. The aim of our work is therefore, to identify small drug-like molecules that bind to the DNA-binding domain of FOXO3 and modulate the expression of target genes.

Methods: We used a combination of different *in-silico* approaches to select 76 substances for further investigation. These compounds were tested via propidium iodide (PI) FACS analysis and substances that prevented FOXO3-induced apoptosis were analyzed further. To verify that the inhibition of apoptosis is due to an inhibition of transcriptional activity, the expression of the 3 FOXO3-specific target genes Bim, SESN3 and c10orf10 has been investigated via RT-PCR.

Results: 21 substances have been shown to prevent FOXO3 induced apoptosis. 2 of the substances, S2 and S9, were already investigated via RT-PCR and both efficiently block the expression of target genes after FOXO3 activation. So, these 2 substances modulate FOXO3 activity in a target gene specific way.

Conclusion: To our knowledge, we are the first to report the identification of compounds that modulate FOXO3 transcriptional activity in a target gene specific manner.

72 Fatty Alcohols Accumulate in Cultured Fibroblasts of Sjögren Larsson Syndrome Patients

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Background: Alkylglycerol monooxygenase catalyses the tetrahydrobiopterin dependent cleavage of ether lipids, producing glycerol derivatives and long chain fatty aldehydes. Fatty aldehydes can also be formed by other lipid pathways and by oxidative stress. To protect cells from damage, fatty aldehydes are readily converted into their corresponding fatty acid by the membrane-bound enzyme fatty aldehyde dehydrogenase. A less abundant pathway in mammalian cells is the formation of fatty alcohols. Impaired fatty aldehyde dehydrogenase function causes the Sjögren Larsson Syndrome (SLS). This autosomal recessive disorder results in the accumulation of abnormal lipid species, including ether lipids and fatty alcohols, in cells and causes symptoms such as ichthyosis, mental retardation and spasticity.

Methods: Here we present a novel approach to study the fate of different lipids species in living cells. Sjögren Larsson Syndrome patient fibroblasts were incubated with pyrene-labeled fatty alcohols, fatty aldehydes, ether lipids and fatty acids. Normal human fibroblasts served as controls. Fluorescent metabolites were quantified after 24h in the supernatant medium of the living cells by the use of HPLC separation und fluorescent detection.

Results: Our experiments showed that the fatty aldehyde was almost completely removed in all cell types. While in control cells mainly the corresponding fatty acid was formed, fatty alcohol was the major product in SLS cells. Incubation with the fatty alcohol substrate showed that it remained unmetabolized in SLS cells, whereas it was converted into the corresponding acid in control cells.

Conclusion: In this work we demonstrate that metabolite profiles of SLS and control cells differ strongly. Lack of fatty aldehyde dehydrogenase function in SLS cells leads to accumulation of fatty alcohols from fatty aldehydes.

73 Identification of performance associated miRNAs and other small non-coding RNA molecules in murine skeletal muscle tissue

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MiRNAs are a small non-coding RNA molecules and act on a post transcriptional level by targeting mature mRNAs and thereby inhibiting their translation process. They are a highly abundant small non-coding RNA class and display fundamental regulators of gene expression. Misregulation of miRNAs can lead to severe disease models and anomalies in organisms.

In this study we want to reveal differentially expressed miRNAs and other small non-coding RNAs in skeletal muscles of endurance trained mice in various training stages. In contrast to microarray based experiments, the use of the ABI SOLiD™ 5500 next generation sequencing system (NGS) allows us to find novel small non-coding RNA molecules, which have not been annotated before. Bioinformatical analysis of the data, obtained by NGS, allows us to find changes in the expression profile of these small non-coding RNAs and will reveal possible targets for differentially expressed or novel small non-coding RNAs. This could lead to further insights in how the muscle deals with endurance training and which mRNAs are effected by the endurance induced changes in expression levels of the different small non-coding RNAs, thus leading to a better understanding of skeletal muscle development.

74 Influence of EBV-encoded Proteins on the Vault RNA Level

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Recently several novel and previously reported non-protein-coding RNAs (ncRNAs) have been identified to be upregulated upon Epstein-Barr virus (EBV) infection in human B-lymphocytes. The common characteristic of all ncRNAs is to exert their functions at the RNA level since they are not translated into proteins. A group of these significantly upregulated ncRNAs are called vault RNAs (vtRNAs). ,b About 5% of the total cellular vtRNAs are connected to the vault particle, the largest known ribonucleoprotein particle (RNP) in eukaryotic cells. However the function of this ncRNA family and moreover of the vault particle remains still rather unclear. Our previous findings suggest a link between EBV infection and vtRNA expression and so consequently the question arises which part of the viral genome is responsible for the upregulation. EBV establishes latency III infections in the investigated Burkitt Lymphoma cell line (BL2), in which additional to two small RNAs (EBER 1 and 2) and several miRNAs (BARTs) nine virally encoded proteins (EBNA-1, 2, 3A, 3B, LP, 3C, LMP-1, 2A, 2B) are expressed.

To address this question we have separately overexpressed specific EBV-encoded, latently expressed proteins in BL2-cells to determine the influence on the vault RNA levels. To this end stable cell lines were created that express these proteins and subsequently the vtRNA expression was analyzed via northern blotting. Thereby we identified one EBV-encoded protein, called Latent Membrane Protein 1 (LMP1), which significantly contributes to the vault RNA upregulation. We used LMP1 mutants to characterize the region of the protein responsible for triggering the elevated vtRNA expression. The applied point mutations were located in the effector domains called C-terminus activator region 1 and 2 (CTAR1 and 2) of LMP1 and are essential for the activation of different signaling pathways in infected cells. Our results suggest, that both regions, CTAR1 and CTAR2, are responsible for LMP1-mediated vtRNA upregulation and that the NF-κB pathway might be involved in this process.

The main goal of this approach is to achieve new insights concerning the functions of the vault RNAs especially in connection with virus propagation.

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75 The chromatin remodelling factor CHD1: roles and functions in ES cells and in early mouse development

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The genetic information contained in the eukaryotic genome and the development of a functional organism are highly dependent on the action of many different factors and complexes that regulate gene expression in an extremely precise and coordinated way. Chromatin represents an important level of gene regulation, and numerous proteins are known to contribute to its continuous and highly dynamic reorganization. CHD1 is a chromatin remodelling and assembly factor that associates with the promoter of active genes and that is known to be important to maintain pluripotency in ES cells (Gaspar-Maia et al., 2010). Furthermore, in flies CHD1 is required for the incorporation of the variant histone H3.3 into the paternal pronucleus during fertilization (Konev et al., 2007). Although CHD1 was the first member of the CHD subfamily of ATPases to be discovered, its function in development is still largely unknown. Currently, we are investigating the role and the mechanisms of regulation of the mammalian ATP-dependent chromatin remodelling factor CHD1 in development by generating a conditional knock-out mouse model. Moreover, the establishment of chd1+/-, chd1-/- as well as CHD1 overexpressing mouse ES cell line allows us, trough the use of techniques like embryoid body generation and selective in vitro differentiation, to analyze the function of this protein during early development and in different cell types. Here, we report on the progress of this work.

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76 Regulation of Depp by the transcription factor FKHRL1

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Background:

Neuroblastoma (NB) is the most common solid tumor in childhood and develops from undifferentiated progenitor cells of the sympathetic nervous system. In neuroblastoma cells an increased activity of the phosphatidylinositol-3 kinase (PI3K) protein kinase B (PKB/AKT) pathway was reported. PKB/AKT regulates among other proteins the transcription factors of the FoxO family, specifically the transcription factor FoxO3 (FKHRL1) and thus growth, cell cycle progression and cell death.

As part of Affymetrix gene-chip analysis for FKHRL1-regulated genes the "decidual protein induced by progesterone" (Depp), also described as fasting-induced gene (FIG), was identified as an FKHRL1-induced gene. The aim of this study is to characterize this FKHRL1-regulated target gene in more detail and to explore possible functions of Depp in neuroblastoma cells.

Methods:

Depp induction by activated FKHRL1 was analyzed at mRNA level using quantitative RT-PCR and at protein level using western blot analysis. For that purpose a Depp-specific antibody was generated. RT-PCR was also used to show that Depp is regulated in an insulin-dependent manner in neuroblastoma cells. In addition a promotor-reporter assay was performed to clarify whether Depp is a direct transcriptional target of the FKHRL1 transcription factor. To learn more about the subcellular localization of Depp retroviral vectors for the expression of N- and C-terminal EYFP-Depp fusion proteins were constructed and infected into neuroblastoma cells. Furthermore, knock-down of Depp using shRNA technology was done to investigate possible functions of Depp during FKHRL1-induced cell death in neuroblastoma cells.

Results:

We were able to show that Depp is regulated by FKHRL1 at mRNA and protein level in different neuroblastoma cell lines. This regulation is affected by insulin levels and depends on direct binding of FKHLR1 to the Depp promoter. Furthermore it could be shown that Depp is mainly localized in the nucleus but gradually translocates to the mitochondria, maybe due to increasing cell density. The knock-down of Depp resulted in decreased apoptosis during FKHRL1-activation, suggesting a possible pro-apoptotic effect of Depp.

Conclusion:

Depp is a direct transcriptional target of FKHRL1 and affects apoptosis in neuroblastoma cells.

77 The Role of Ornithine Supply in Siderophore Biosynthesis in *A. fumigates*

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Background: Iron is an essential nutrient required for a wide range of cellular processes. However, excessive iron is toxic. Therefore, microorganisms evolved fine-tuned iron uptake and storage mechanisms, such as the siderophore system. The opportunistic fungal pathogen *A. fumigatus* produces siderophores (low-molecular mass iron-specific chelators) to acquire, store and distribute iron. Past studies indicated coordination of siderophore biosynthesis with supply of its precursor ornithine.

Methods: The role of mitochondrial ornithine production in siderophore synthesis of *A. fumigatus* was characterized by analysis of the phenotypic consequences of genetic inactivation of the putative mitochondrial ornithine exporter, AmcA (Afu_8g02760).

Results: Inactivation of AmcA resulted in a decrease in the cellular ornithine content as well as a decrease in siderophore production. In the presence of the iron chelator bathophenanthroline disulfonate, which inhibits siderophore-independent iron uptake, AmcA-deficiency decreased conidiation indicating increased iron starvation. AmcA-deficiency however did not affect the cellular content in polyamines, which are also derived from ornithine via ornithine decarboxylase. Nevertheless, AmcA-deficiency increased the susceptibility of *A. fumigatus* to eflornithine, an inhibitor of ornithine decarboxylase, most likely due to a decreased ornithine pool.

Conclusion: Siderophore synthesis is mainly fueled by mitochondrial production of ornithine, rather than by conversion of arginine to ornithine in the cytoplasm. There exists coordination between siderophore synthesis and its precursor supply. This study also indicates a prioritization of ornithine flux into synthesis of polyamines compared to siderophores, emphasizing the essentiality of polyamines.

78 CHD1 contributes to *Drosophila* immunity

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Background: Eukaryotic chromatin is structured in a way that allows dynamic DNA processing in accordance with maximum DNA condensation. The ATP-dependent motor protein CHD1 participates in the assembly and remodelling of chromatin and functions as a regulator of transcription at global and gene-specific levels.

In order to further characterize the biological functions of *Drosophila* CHD1, we have performed gene expression profiling of *Chd1*-mutant versus wild-type larvae. Here we show that a considerable fraction of genes that were misregulated in the absence of CHD1 were functionally related to immune response, detoxification and stress response in the fly.

Methods: To examine the biological consequences of the observed misregulation, we tested the susceptibility of *Chd1*-deficient flies to local or systemic infections with different classes of microbes. Furthermore we employed RT-qPCR to examine whether misregulation of any of the major *Drosophila* immune response pathways (Imd, Toll, JAK/STAT, JNK) could be linked to increased susceptibility of *Chd1*-deficient flies to infection.

Results: We found that *Chd1*-deficient flies are highly sensitive towards gut-specific infection by the gram-negative bacteria *Pseudomonas aeruginosa*. Infections with gram-positive *Staphylococcus aureus* caused a moderate decrease of fly viability, while a fungal pathogen, *Rhizopus oryzae*, had no specific effect on *Chd1*-mutant flies. RT-qPCR analyses revealed that JNK and JAK/STAT pathway target genes showed only moderate transcriptional changes, whereas Imd-mediated expression of antimicrobial peptide genes was strongly upregulated in the *Chd1* deletion mutant. Thus suggesting that CHD1 might act as a negative regulator of the gut-specific Imd-signaling pathway.

Conclusion: We present evidence that CHD1 acts as a regulator of the Imd-signaling pathway, potentially by attenuating immune responses to prevent damage due to sustained overactivation.

79 1.4. IAP-antagonists from natural resources

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Background:

Inhibitor of apoptosis proteins (IAPs) are proteins that bind caspases and thereby interfere with apoptotic cell death signaling via death receptors or intrinsic cell death pathways. In addition modulators of apoptosis such as Smac/Diablo and caspase-independent apoptosis pathways are inhibited by IAPs making these apoptosis-inhibitors to attractive targets for anti-cancer therapy. Several IAPs carry a RING domain and act as E3-ubiquitine ligases that mark their binding partners for ubiquitination and degradation. XIAP which is the most potent inhibitor of apoptosis among the IAP family directly binds caspase 9 via its BIR3 domain. Other IAPs such as cIAP1 and cIAP2 act indirectly by sequestering Smac/Diablo away from XIAP. The XIAP protein is frequently overexpressed in human leukemia and tumors of prostate and breast.

Methods:

The aim of the project is to identify novel cell-permeable small molecules from natural resources that bind and inhibit IAPs. The target-oriented search for bioactive natural products is achieved by an in silico guided approach. Proposed natural ligands of the BIR3 domain are analyzed in their molecular and physiological mode of action in vitro and in vivo by fluorescence polarization assay (FP), protein fragment complementation assay (PCA) and propidium iodide-FACS-analysis (PI-FACS).

Results

So far we screened 100 predicted substances by FP-assay. Out of this group 30 substances could be identified that show a positive effect in binding to the recombinant BIR3 domain of XIAP. These substances were then further investigated in two different cell-based assays, PI-FACS-analysis and PCA-assay. PI-FACS-analysis revealed that 10 out of the 30 substances sensitized XIAP overexpressing cells to apoptosis induction. These 10 substances were also active in PCA analyses suggesting that these compounds efficiently enter the cells.

Conclusion:

The substance EHES1SP50 is the most active compound and will be further investigated.

80 Regulation of FOXP1 by FoxO3a/FKHRL1 in human neuroblastoma

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Background: In neuroblastoma the PI3K/PKB signaling pathway is hyperactive and leads to inactivation of the transcription factor FoxO3a/ FKHRL1, to chemotherapy resistance and to survival of neuroblastoma cells. Via Affymetrix chip analysis we identified Forkhead Box P1 (FoxP1) as a target gene of FKHRL1. FoxP1, an important transcriptional repressor is a member of the FOX family of transcription factors and plays an important role in cardiac, lung and lymphocyte development.

Methods: FKHRL1 mediated regulation of FoxP1 was analyzed via real time RT-PCR and immunoblot in transgenic FKHRL1 expressing neuroblastoma cells. To analyze whether FKHRL1 directly activates the FoxP1 promoter a luciferase reporter assay was performed. A tetracycline regulated FoxP1 expression plasmid was constructed and retrovirally infected into SH-EP cells to determine the effects of FoxP1 on cell cycle regulation and apoptosis induction. Apoptosis induction was measured by propidium iodide – FACS – analysis. The regulation of involved proteins is determined by immunoblot analysis. Furthermore FoxP1 was knocked down by short hairpin RNA to determine its role in FKHRL1 induced apoptosis in neuroblastoma.

Results: We found that FoxP1 mRNA increases within 3 to 6 hours and FoxP1 protein levels were elevated within 16 hours after FKHRL1 activation in all investigated cell lines. The FoxP1 promoter is efficiently activated by FKHRL1 suggesting that FoxP1 is a direct target of FKHRL1. Tetracycline – regulated expression of FoxP1 in SH-EP cells significantly decreases proliferation. Furthermore ectopic FoxP1 expressing cells show reduced sensitivity to doxorubicin.

Conclusion: Our results demonstrate that FoxP1 is a direct target of FKHRL1 and plays a role in cell cycle progression and drug resistance.

81 In Vivo and in Vitro Analysis of Protein Domains of the ATP-dependent Chromatin Assembly Factor CHD1 in *Drosophila melanogaster*

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CHD1 was identified as ATP-dependent chromatin assembly factor containing an ATPase subunit that belongs to the SNF2 superfamily of proteins. The SNF2-like ATPase domain is located in the central region of the protein and its function facilitates the alteration of chromatin structure by disrupting and mobilizing nucleosomes. Based on the presence of other conserved domains, the remodeling enzymes are classified into different subfamilies. CHD1 is characterized by tandem chromodomains in the N-terminal region, which were shown to bind H3K4me3 in human CHD1, but were less selective in *Drosophila* CHD1. It is believed that CHD1 is targeted to regions of active transcription by this interaction.

However, our work in *Drosophila* indicates that the chromodomains are not required for the chromatin localization. To examine whether the DNA-binding domain is important for the correct localization of CHD1, we investigated the function of this domain, which is located in the C-terminal part of the protein. For in vivo analysis a fly-line lacking the DNA-binding domain of the CHD1 protein was generated. Preliminary data of these flies indicate that the DNA-binding domain seems to be partially necessary for the localization of CHD1 at regions of active transcription. Additionally, it also plays a role in fertilization process and spermatogenesis in *Drosophila.*

For in vitro analysis we generated different mutant proteins that either lack the C-terminus completely, partially, or have mutations in the DNA-binding domain. These proteins are used for chromatin assembly and nucleosome remodeling activity experiments and compared to the full-length CHD1.

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82 mTOR Activity in $CD8^+$ T Cells is Dependent on Differentiation Stage and Stimulation

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Background: With age, the immune system undergoes significant changes, termed “immunosenescence”, which are the basis for the increased frequency and severity of infectious diseases and decreased protective effect of vaccinations in the elderly. The loss of the costimulatory molecule CD28 on $CD8^+$ T cells is one of the most prominent biological indicators of aging in the human immune system. $CD28^-$ $CD8^+$ T cells have been shown to accumulate in with age and are believed to contribute to the development of age-related diseases due to their proinflammatory activity. The conditions under which these cells survive or die are therefore a matter of interest. mTOR (mammalian target of rapamycin) is a central regulator of metabolism and aging and occurs as two functionally and structurally distinct multiprotein complexes termed mTOR complex 1 (mTORC1) and mTORC2. To define their role in $CD8^+$ T cells we analyzed mTOR signaling in $CD28^+$ and $CD28^-$ CD8+ T cells.

Methods: CD28+ and CD28- CD8+ T cells were isolated from healthy donors and stimulated either via the T cell receptor or with the homeostatic cytokine IL-15. To analyze the mTOR pathways, we performed Western Blots of mTOR readouts.

Results: Following antigenic stimulation, mTOR activity was significantly lower in $CD8^+$ $CD28^-$ T cells as compared to their $CD28^+$ counterparts. In contrast, upon homeostatic stimulation of $CD8^+$ T cells with the cytokine IL-15 mTOR activity was higher in the $CD28^-$ subset.

Conclusion: We demonstrate that $CD8^+$ T cells lose their ability to activate mTOR signaling upon antigenic stimulation along with differentiation. In contrast, the capability of $CD8^+$ T cells to respond to the homeostatic cytokine IL-15 in terms of mTOR activity increases with differentiation. We therefore propose a model of mTOR activity in CD8+ T cells as a function of stimulation and differentiation and conclude that mTOR activity in CD8+ T cells greatly depends on their differentiation stage as well as on their environment.

83 Upregulation of miR-24 is associated with a decreased DNA damage response upon etoposide treatment in highly differentiated CD8+ T cells sensitizing them to apoptotic cell death

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Abstract

The life-long homeostasis of memory $CD8^+$ T cells as well as persistent viral infections have been shown to facilitate the accumulation of highly differentiated $CD8^+CD28^-$ T cells, a phenomenon that has been associated with an impaired immune function in humans. However, the molecular mechanisms regulating homeostasis of $CD8^+CD28^-$ T cells have not yet been elucidated. In this study, we demonstrate that the miR-23~24~27 cluster is up-regulated during post-thymic $CD8^+$ T cell differentiation in humans. The increased expression of miR-24 in $CD8^+CD28^-$ T cells is associated with decreased expression of the histone variant H2AX, a protein that plays a key role in the DNA damage response (DDR). Following treatment with the classic chemotherapeutic agent etoposide, a topoisomerase II inhibitor, apoptosis was increased in $CD8^+CD28^-$ when compared to $CD8^+CD28^+$ T cells and correlated with an impaired DDR in this cell type. The reduced capacity of $CD8^+CD28^-$ T cell to repair DNA was characterized by the automated fluorimetric analysis of DNA unwinding (FADU) assay as well as by decreased phosphorylation of H2AX at Ser139, of ATM at Ser1981 and of p53 at Ser15. IL-15 could prevent etoposide-mediated apoptosis of $CD8^+CD28^-$ T cells, suggesting a role for IL-15 in the survival and the age-dependent accumulation of $CD8^+CD28^-$ T cells in humans.

84 Influence of Nano-Crystalline Diamond Implant Coating on Connective Tissue Healing. An *in vivo* Study.

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Background:
Skin is not able to repair itself with a restituito ad integrum. Thus soft tissue in contact to percutaneous implants is characterized by tight and dense scar formation. Scar is a fibrous connective tissue with a limited blood supply and reduced immunological response. Thus the scar at the interface between adjacent to the implant is prone to bacterial invasion resulting in infection. Sealing this interface via improving soft tissue adhesion to the implant will restore the role of the skin as a barrier against bacteria. It has been demonstrated that a certain surface roughness can influence soft tissue attachment. Moreover coating titanium with nano-cystalline diamond (NCD) can improve implant resistance to bacterial colonization. The aim is to investigate, besides the roughness, the influence of different terminated NCD coating on soft tissue adhesion to the implant.
Methods:
Discs constituted by polished pure titanium (as a control group) and further coated with hydrophilic NCD (O-NCD) and hydrophobic NCD (H-NCD) were inserted into the subcutaneous connective tissue of 24 male Wistar rats. 1 and 4 week after operation samples were evaluated via histology (Van Gieson staining) and immunohistochemistry (integrin α_5 and TNF-α).
Results:
Histology showed that O-NCD could generate a more loose soft tissue compared to tight scar, which in turns was present at the H-NCD and pure titanium. Immunohistochemistry revealed an increased expression of Integrin α_5 indicating an improved adhesion to the hydrophilic surface after 1 week. At the same time inflammation marker TNF-α was decreased compared to the other groups.
Conclusion:
Coating titanium with hydrophilic NCD can positively influence soft tissue adhesion leading to an increased number of cell and a reduced inflammatory response. This *in vivo* model permits the investigation of soft tissue/implant interface and can be useful for further studies on cell attachment to different coated surfaces.

85 Cell Cycle Distribution of HPV-16 E7 in CaSki Cells

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Background: High-risk human papillomaviruses (HPV) are the main cause for cervical cancer. E7 is the major HPV oncoprotein. Although many biochemical functions of E7 have been described in recent years, the subcellular localization of E7 is not well described yet. In this study we employed the anti-HPV-16 E7 antibody, RabMab42-3, which recognizes a conformational epitope in the E7 carboxy-terminal zinc-finger, to more precisely investigate the subcellular localization of endogenous HPV-16 E7 oncoprotein in the cervical cancer cells, CaSki.
Methods: Indirect immunofluorescence was carried out as described in Dreier et al., 2011; Co-immunoprecipitation and determination of protein stability as described in Morandell et al., 2008.
Results: We show that E7 is a predominantly cytoplasmic protein; however, a significant amount of E7 is localized in the nucleus in a cell cycle dependent manner. Nuclear E7 is well detectable in mid-G1 and at S/G2-phase transition but not in early G1, late G1 and S-phase. Moreover, using indirect immunofluorescence confocal microscopy, co-immunoprecipitations and half-life measurements we demonstrate that the physical and functional interaction between E7 and the retinoblastoma protein strongly differs during the cell cycle. Additionally, the colocalization of E7 with other cellular E7-target proteins, such as γ-tubulin and F-actin was investigated and new targets, such as the nuclear pores, the endoplasmic reticulum (ER) and α-tubulin were identified.
Conclusion: Our findings suggest that HPV-16 E7 is localized in several distinct subcellular compartments in a cell cycle dependent manner. Presumably E7 is imported into and exported from the nucleus over the course of the cell cycle by shuttling through the nuclear pores. We demonstrate a predominant interaction of E7 with Rb in the M phase, leading to the degradation of Rb. Additionally, we show the colocalization of E7 with α-tubulin, γ-tubulin, F-actin and the endoplasmic reticulum.

86 Hyaluronan and its role on the stemness of human bone-derived mesenchymal stem cells

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The unbranched polysaccharide Hyaluronan (HA) represents a major component of mesenchymal extracellular matrices. HA is highly hydrated and thus functions as a biological lubricant; it regulates water balance in the interstitium and thus determines tissue volume; it creates space for molecular transport and facilitates cell migration; yet it is also recognizes receptors such as CD44 which in turn guide cell fate decisions such as cell migration, proliferation and differentiation.

Here we show that the niche of mesenchymal stem cells (MSC) comprises HA, which has an direct impact on MSC stemness as assessed by examination of colony-formation, long term proliferation capacity, differentiation assay, and expression of stemness factors.

HA positively affects MSC stemness by means of an increased proliferation and osteogenic differentiation capacity. Age related changes in the expression level of HA metabolism associated genes could therefore represent one reasonable explanation for the prevalence of age-associated adipositas within aging bone.

87 DC-like Cell-dependent Activation of Human Natural Killer Cells by the Bisphosphonate Zoledronic Acid is Regulated by γδ T Lymphocytes

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Background: Bisphosphonates are mainly used for the inhibition of osteoclast-mediated bone resorption but also for the treatment of metastatic bone disease. Because, in addition to their effects on bone metabolism, bisphosphonates may have immunomodulatory potential, we tested the effects of bisphosphonate administration on innate lymphocytes.

Methods: We treated IL-2 primed human $CD56^+$ peripheral blood mononuclear cells with the bisphosphonates zoledronate and pamidronate. Over several days we checked for lymphocyte activation and tested for the production of T_H1 and T_H2 related cytokines using a flow cytometric bead array.

Results: The bisphosphonate zoledronate could induce IFN-γ production not only in γδ T cells but, surprisingly, also in natural killer (NK) cells in a manner that depended on antigen-presenting cells, which share properties of inflammatory monocytes and dendritic cells (DC-like cells). In the presence of γδ T cells, DC-like cells were rapidly eliminated, and NK cell IFN-γ production was silenced. Conversely, in the absence of γδ T cells, DC-like cells were spared, allowing NK cell IFN-γ production to proceed. γδ T cell–independent NK cell activation in response to zoledronate was due to downstream depletion of endogenous prenyl pyrophosphates and subsequent caspase-1 activation in DC-like cells, which then provide mature IL-18 and IL-1β for the activation of IL-2–primed NK cells. Inhibition of caspase-1 almost abolished IFN-γ production in NK cells and γδ T cells, indicating that caspase-1–mediated cytokine maturation is the crucial mechanism underlying innate lymphocyte activation in response to zoledronate.

Conclusion: Zoledronate not only induces γδ T-cell but also NK cell activation. The effect strictly depends on DC-like cells and is regulated by γδ T cells. In clinical studies of zoledronate-based cancer therapy, immune monitoring should therefore not only focus on γδ T cells but also on NK cells.

88 The effect of immunosuppressive drugs on immune cells from young and elderly persons

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Life expectancy as well as the average age of patients undergoing dialysis increases constantly. Consequently, renal transplantation with consecutive immunosuppressive therapy is no longer limited to young recipients with terminal renal failure. In the present project we investigate how different types of immunosuppressive therapy, namely the calcineurin inhibitors Cyclosporine A (CsA) and Tacrolimus (FK506), as well as the mTOR-inhibitor Rapamycin affect T cell function in young and elderly persons, in vitro.
The effect of immunosuppressive drugs on proliferation, cell viability, cytokine production (IL-2, IFN-g), telomere length and H2O2 production of human peripheral blood mononuclear cells (PBMCs) isolated from whole blood of young (n=10; median 27) and old (n=12; median 71) donors was analyzed in vitro. PHA was used as activating stimuli.
The inhibition of proliferation was decreased in PBMCs isolated from elderly donors, especially after incubation with Rapamycin. All three immunosuppressive drugs inhibited the production of IL-2 equally well, whereas the production of IFN-g was less well inhibited by Rapamycin. Cyclosporin A and Tacrolimus had a stronger cytotoxic effect on cells from elderly persons. Both calcineurin inhibitors induced H2O2 production and led to a shortening of telomeres. In the case of Tacrolimus the induction of H2O2 production as well as the reduction of telomere length was less pronounced in old donors. Rapamycin had no effect on H2O2 production and telomere length.
Our results demonstrate that the effects of immunosuppressive drugs on PBMCs differ between young and elderly persons. Specific treatment regimes for the elderly might therefore be considered.

89 The Role of FAHD1 in Cellular and Organismic Aging

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Background: The 25 kDa protein FAHD1 (FAH domain containing protein 1) is the second member of the FAH superfamily that has been identified in the human genome but it has not been researched extensively until recently. Using a proteomic screen for mitochondrial proteins that are differentially regulated in young and senescent human cells, FAHD1 has been identified as a target for age-related post-translational modifications. In a recent study, the potential enzymatic activity as well as the tissue-specific and subcellular localization of the protein was clarified (Pircher *et al.*, 2011).
Methods: The physiological function and regulation of FAHD1, as well as the nature and regulatory mechanisms of FAHD1 interaction partners will be clarified by a combination of human cell culture work and work in the model organism *C. elegans*.
Results: Concerning organismic aging, it has been shown that the depletion of the FAHD1 gene product by RNAi led to a significant reduction in the mean lifespan of *Caenorhabditis elegans*. During these experiments the RNAi phenotype in respect to embryonic and larval development, growth, fertility, brood size, morphology, behavior, and locomotion was analyzed, but no abnormalities compared to wild-type worms were observed. To further examine the physiological function of the FAHD1 ortholog in the worm a FAHD1 knock-out mutant *C. elegans* strain was generated. This mutant worm shows abnormalities in its phenotype compared to wild-type worms and will be used in metabolic studies and epistasis experiments.
Conclusion: The findings suggest an important function for the FAHD1 ortholog in *C. elegans*. It is expected that the functional characterization of the gene product will provide new insight in the metabolism of eukaryotic cells.

90 Simulation On Handling Instruments In The Femoral Canal

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Background: Removing bone - during primary and revision total hip arthroplasty (THA) - or cement in revision THA is a necessity in the preparation of the femoral canal. Inserting straight tools in a 3D curved cavity have a limit and are only able to reach areas in a given depth, depending on the size and shape of the canal and its proximal opening. The aim of this study was to determine how deep we can reach with straight instruments into the femur.

Methods: The geometry of the femoral canal was used to simulate the access of a virtual instrument. The osteotomy was extended laterally, in order to gain deeper access to the femoral canal. The ultimate accessible depth, the depth at which 100% of the layer was accessible and the depths for intermediate steps in 10% intervals were determined. To test which depths could be reached with instruments of various diameters the One- Way ANOVA was used. To calculate the influence of possible extensions of the access portal a repeated-measures ANOVA was used.

Results: For the normal osteotomy, in average the ultimate depth, which could be reached by the virtual instrument was between 33.9 cm ± 3.4 cm for a diameter of 1 mm, and 20.0 cm ± 1.2 cm for a diameter of 10 mm. The average depth at which 100% of a cross section of the femoral canal could be reached was 10.1 cm ± 1.2 cm and 5.7 cm ± 0.7 cm respectively. Already a lateral extension of the osteotomy of 2.5 mm enabled a significant deeper access ($p \le 0.001$), independent of the diameter of the instrument.

Conclusion: Considering the length of an average hip prosthesis and an additional space for bone cement, it can be assumed that a revision cannot be accomplished without extending the osteotomy, windowing the femur or using a special angulated instrument. The results show, that a small lateral extension of the osteotomy by 2.5 mm already has a significant influence ($p \le 0.001$) on the reachable depth where 100% of a cross section can be accessed for all calculated diameters.

91 Moonmilk – Mineral Forming Microbes

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Background

Moonmilk terms white, plastic calcite deposits, which can be found in caves all around the world. Despite the fact that the Swiss Conrad Gessner already described this secondary mineral deposits in 1555, moonmilk was put into focus of microbiology for the first time about 20 years ago, when it was recognized that microorganisms are associated with it. However, only fragmentary data are available and little is known about the functionality of this extreme, oligotrophic habitat. This study is the first attempt to investigate moonmilk-inhabiting microbes in an Alpine cave in Austria.

Methods

In October 2010 samples of different moonmilk deposits were taken from an isolated part of the Hundalm cave (Tyrol) under aseptic conditions and stored frozen. To get an insight into the microbial community structure we tried to cultivate microbial members on seven different media. Eight grown pure cultures as well as 14 moonmilk samples were analyzed by molecular-biological techniques, including PCR/DGGE and sequencing.

Results

Prokaryotic cultivation could only be achieved on a complex medium, but not on media designed for oligotrophic organisms. Genomic DNA could be detected in ten out of 14 different samples. An astonishing diverse bacterial and archaeal community with high abundances was proved. In none of the samples fungi could be detected. Among the sequenced organisms were *Bevundimonas subvibrioides*, *Flavobacterium omnivorum* and *Sporosarcina macmurdoensis*.

Conclusion

With the present study it could be proved that a highly diverse, complex bacterial and archaeal community with high abundances inhabits moonmilk in the Alpine Hundalm cave. These preliminary data go conform with various studies from other countries and climate zones. Further investigations shall reveal a bigger part of the microbial composition, variations within different deposits, expose the nutrient and energy sources and lead to a better understanding of this fascinating geogenic ecosystem.

92 Photoacoustic imaging based analyses of pancreas regeneration in adult zebrafish

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In all vertebrates β-cells are the only natural source of the blood-sugar regulating peptide hormone insulin and in humans the loss or dysfunction of β-cells leads to diabetes. We focus on the characterization of endogenous programs of β-cell regeneration existing in different vertebrates organisms, but for which neither the cellular mechanisms nor the molecular pathways have been characterized so far.

Zebrafish embryos are small and transparent and therefore they are a favored model for microscopic analyses of embryonic β-cell formation and regeneration. However, mechanisms of β-cell regeneration most similar to those observed in mammals have only been reported in older zebrafish. A major limitation for studying the in vivo processes underlying β-cell regeneration in older fish is the optical inaccessibility of the pancreas in the middle of the abdomen. We use transgenic reporter lines to establish and improve detection of deeply located fluorochromes by multispectral photoacoustic imaging (PAI) as a method to visualize β-cells in juvenile and adult fish. Further, we generate novel PAI-optimized transgenic fishlines to enable fast high-resolution analyses of pancreatic cell types in adult zebrafish.

Currently, the sensitivity of the PAI setup needs some improvements to meet the requirements for proper detection of fluorochromes in living cells. By using a transparent zebrafish mutant the signals emerging from pigmented cells of wildtype zebrafish were reduced in PAI scans. The establishment of a reliable method providing the possibility of timewise precise induction of pancreatic ß-cell ablation is still in progress.

Finally this methodology will be employed in an in-vivo beta-cell ablation/regeneration model to study the cellular dynamics of β-cells regeneration in the adult zebrafish. The aim is to improve the methodology for efficient generation of insulin producing β-cells to facilitate on new approaches to cell replacement therapies for treating type I diabetes.

93 The use of Fatty Acid Methyl Ester Profiles to Investigate Reactor Performance in Anaerobic Digestion

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Background:

Biogas is an interesting green energy source, which can be produced out of a wide variety of biological materials. The use of municipal solid waste as feedstock offers the advantage that no potential foodstuffs have to be used and is also a very effective way of waste treatment. However, the anaerobic digestion process is very complex and the interactions of the involved organism are not clearly understood. In the presented study we used phospholipid fatty acid profiles (PLFA) to investigate the involved community

Methods:

For the determination of PLFA profiles the samples were extracted with Chloroform / Methanol. The chloroform phase, containing the lipids, was separated by solid phase extraction in to the major lipid classes. The phospholipid containing methanolic fraction were transesterified in methanolic HCl. The so formed fatty acid methyl esters were extracted with hexane and analysed by GC. The reactor performance parameters were regularly monitored by the plant staff

Results:

During this investigation we monitored a 750 m³ biogas reactor (Roppen/Tyrol) over a period of six months. Within this period we could observe four different phases regarding the gas production: low (I), increasing (II) high (III) and decreasing (IV) gas production. According to these phases we also were able to find considerable changes within the PLFA profiles.

Conclusion:

The drastic variations in the reactor performance should lead to alterations in the microbial community and indeed, by the use of PLFA profiles we were able to track them. We found, that the community changes very quickly as a consequence of increased nutrition availability. But interestingly the changes in the microbial community are quite moderate, if nutrients are reduced. These findings clearly show that community structure of this anaerobic digestion is quite robust to nutrition stress and that PLFA analysis are well suited to investigate microbial communities within complex habitats.

94 The role of islet genes during formation of zebrafish endocrine and exocrine pancreas

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The LIM homeobox protein Islet1/Isl1 belongs to a set of highly conserved transcription factors that are required for the development of the pancreas, which is a vertebrate specific organ of the digestive system with essential functions in food digestion and glucose homeostasis. During pancreas development isl1 is expressed in postmitotic progenitors of all endocrine cells and in mesenchyme neighboring the pancreatic endoderm. Studies of isl1 mutant mice suggested that expression in these cells correlates with essential functions in endocrine cell differentiation and formation of mesenchyme that is required to induce exocrine tissue. However, analyses on pancreatic function were very limited in the isl1 mutant mice due to the early lethality around embryonic day 10. Here we present the characterization of pancreatic phenotypes of isl1 mutant zebrafish. Importantly, loss of isl1 in zebrafish does not lead to early embryonic lethality and therefore these mutants also enables studies on late embryonic phenotypes that were not possible in isl1 mouse mutants. In contrast to what has been suggested based on the isl1 knock out, we find that loss isl1 in zebrafish does not lead to the loss of endocrine cells. Our data rather show that isl1 has different and cell- type specific functions in early and late forming endocrine cells, with only the latter giving rise to mature hormone- producing cells of the adult organism. Similarly, a recent study in mouse using conditional knock out of pancreatic isl1 also revealed cell type specific function of isl1 during later phase of endocrine differentiation. In addition, we show that in zebrafish mesenchyme isl1 has overlapping functions with its paralogous isl2a during non- cell autonomous induction of exocrine tissue. In particular, we find that genetic or anti- sense morpholino based loss of isl1 or isl2a has mild effect on exocrine pancreas formation while the loss of both islets leads to a strong reduction of exocrine tissue without affecting formation of the mesenchyme structures. In summary our data reveal novel insights on islet gene function during endocrine and exocrine cell type and tissue specification and they suggest that islet genes have slightly different functions in mammals and fish.

95 Local Dynamics in Serine Protease Recognition

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Background

Proteases catalyze cleavage of peptide bonds and are vitally important in a wide range of fundamental cellular processes. Far more than 500 proteases have been identified in the human genome individually tied to a unique cleavage pattern. These patterns reach from specificity for a single peptide to broad spectra of cleaved peptides.

Methods

To analyze the impact of local dynamics on protease specificity, subpocket-wise protease specificity scores are presented for a series of homologous chymotrypsin-like serine proteases based on cleavage data from the MEROPS database [1]. These specificity scores appear to be linked to local flexibility of the binding site region [2]. Consequently, B-factors from X-ray structures as well as all-atom 100ns molecular dynamics trajectories using the AMBER package [3] are compared in respect to specificity.

Results

Although all members of the chymotrypsin-like serine protease family share a common tertiary fold, local dynamics patterns observed from X-ray and molecular dynamics B-factors differ between individual proteases. Analyses of specificity and flexibility patterns reveal a weak but general correlation of binding site rigidity and specificity of serine proteases.

Conclusion

As increased flexibility is paralleled by a broader conformational space, a general mechanism of conformational selection [4] in the binding process of serine proteases is proposed. This finding is in agreement to a binding mechanism of snake venom metalloproteases revealed by molecular dynamics simulations [5].

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96 Native chemical ligation of non-hydrolyzable RNA-cysteine conjugates

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Background: Non-hydrolyzable 3'-peptidyl RNAs represent valuable probes for structural and functional studies of the ribosome, especially ribosomal antibiotic resistance. The ribosomal elongation of the peptide chain takes place at the peptidyl transferase center (PTC) of the ribosome. Thereby, the growing peptide chain has to pass through the ribosomal exit tunnel before it can leave the ribosome. Macrolide antibiotics bind at the entrance of this tunnel and thus prevent elongation of the peptide chain.[1] However, bacteria can show resistance by translating short peptides that are highly conserved in their sequence. It is hypothesized that the macrolide interacts in a specific manner with the resistance peptide and thus is expelled from the PTC so that the ribosome is available for protein synthesis again. In this context, non-hydrolyzable 3'-peptidyl-RNAs would represent valuable probes to study the interaction between macrolide antibiotic and resistance peptide in the PTC and the ribosomal exit tunnel.

Methods: Based on a novel approach for the reliable solid-phase synthesis of non-hydrolyzable 3'-peptidyl RNAs[2] we present the synthesis of 3'-cysteinyl RNAs and demonstrate first examples of native chemical ligation (NCL) of 3'-cysteinyl RNAs with peptide thioesters.[3]

Results: Here, we present the synthesis of a series of RNA-3'-NH-peptide conjugates whose peptide moiety represents a sequence that confers resistance to a macrolide antibiotic.[1] Furthermore, we could show by means of ribosomal binding assays that these 3'-peptidyl tRNA mimics bind to the P-site of the ribosome.

Conclusions: This approach permits the synthesis of RNA-peptide conjugates with amino acids, such as arginine, possessing reactive functional side chain groups, which are otherwise difficult to introduce.

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97 Impact of Tetramerization on Neuraminidase Dynamics and Binding Site Conformations

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Background: Influenza neuraminidase (NA) is a tetrameric surface protein of the influenza virus and the target for antiviral drugs e.g., oseltamivir and zanamivir. The conformational diversity of the 150-loop was revealed by crystal structures of the group 1 neuraminidases [1] and investigated by molecular dynamics (MD) simulations [2,3]. The open state conformation shows an additional sub-pocket (150-cavity) exploitable for drug design [4,5].

Methods: We present a systematic analysis of three neuraminidases (avian 2005, pandemic 1918, pandemic 2009) with all-atom, explicit solvent MD simulations applying the Amber forcefield ff99SB.

Results: Comparative simulations of monomeric, dimeric and tetrameric systems show, that the sampled conformational phase space for the tetramer is distinctable from the monomer simulations. We demonstrate, that interactions with adjacent neuraminidase subunits alter the dynamics of the 150-loop.

Conclusion: These results underline the importance of protein-protein-interactions in the neuraminidase tetramer for the examination of molecular flexibility. In consequence, considering these interactions is crucial for drug development and elucidating the mechanism of drug resistance.

98 Prediction of NMR Order Parameters from Molecular Dynamics Simulations

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Background

Flexibility in biomolecular function is a topic of intensive research. It has been established, that flexibility plays a crucial role for a variety of biochemical processes.[1] Molecular Dynamics (MD) computer simulations as well as Nuclear Magnetic Resonance (NMR) spectroscopy are suitable techniques to study biomolecular dynamics at atomic resolution.[2][3]

Methods

We present a method to predict NMR backbone order parameters for the KIX domain[4] form MD simulations by emploing a probabily density based entropy measure. From the states sampled by MD simulations, we obtain a contiuous probability density function of state space by Kernel Desnity Estimation.[5] Subsequent numerical integration over p(t) ln p(t) yields entropy in units of k_B where p denotes the probability density function of the state vector t.[6]

Results

We predict backbone N-H S^2 order parameters for the backbone of the KIX domain. Correlation to the experiments is evaluated by normalizing the obtained entropy from 0 to 1 and subseqent calculation of linear correlation coefficients of normalized entropy and experimental measurements.

Conclusions

The highly flexible nature of the KIX system necessitates the use of enhanced sampling methods to cover conformational dynamics by simulations. Furthermore, the protonation state of the system, set as an initial condition, influences the calculation results significantly. It would be desirable to have structural information that includes the protonation state.

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99 Identification of novel Nox4 Inhibitors from Food Plant Compounds

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Background: NOX4 is a member of the family of NADPH Oxidases whose major known function is the production of 'reactive oxygen species' (ROS) which are considered to be the source of mutagenesis and gross cellular damage. Deregulation of Nox4 and altered levels of ROS have been related to various forms of cancer and other diseases, especially in the field of hypertension and vascular diseases but also to diabetic nephropathy, Alzheimer's disease and Parkinson's disease.
Knockdown of NOX4 has been shown to extend the lifespan of HUVEC in our group. All inhibitors currently available are either not fully characterised, not proven to inhibit NOX4 directly, or toxic. To date nothing is known about the mechanism of Nox4 inhibition, therefore the search for potent NOX4 inhibitors is an important task to learn about the role of NOX4 in the cell.

Methods: A variety of food plant compounds was analysed in cell based assays for Nox4 inhibition, thereby minimising toxic effects in the first place. In line with suggestions from literature we set up a system to screen for, identify and characterise inhibitors at the basis of Chemoluminescence, DHE-staining and Hoechst-Staining. The results were used as a basis for a virtual screening approach that supplied us with new input substances for the screening

Results: Out of approx. 250 substances we identified and characterised a number of substances that reveal an inhibitory effect on Nox4. Characterisation in respect to Nox2, Nox5 and ROS scavenging capabilities was performed and revealed different characteristics.

Conclusion: Since Nox4 deregulation has been implicated in various human diseases, specific inhibition of Nox4 has drawn attention from the field of pharmaceutical development. The results of the screening show substances of high performance with respect to Nox4 inhibition and compare compounds already known in literature to our findings. Also, substances are characterised in terms of specifity for Nox4 relative to the related enzymes Nox2, which is important for immune response, and Nox5. Further experiments, including virtual screening and a NMR approach to determine the structure of the dehydrogenase domain are planed to improve the data on hand.

100 Converting RNA into radicals in the gas phase

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Background

Top-down mass spectrometry (MS) of larger RNA is challenging. Conventional approaches use so-called 'slow-heating' methods such as collisionally activated dissociation (CAD) or infrared multiphoton dissociation (IRMPD) to fragment and sequence even electron RNA ions in the gas phase. These methods, however, can be severely limited by unwanted base losses unless the precursor ion charge is low (high m/z values), for which instrument performance is generally lowest.

In top-down MS of proteins the method of choice is electron capture dissociation (ECD), which relies on radical ion chemistry involving highly charged precursors and provides extensive sequence coverage.

For RNA, ECD has not yet been demonstrated, but radical ions can also be produced by electron detachment dissociation (EDD). In a recent study we have demonstrated nearly full sequence coverage in EDD of RNA of up to 34 nucleotides (nt). Here we extend the methodology to highly modified, larger RNA (tRNA).

Methods

MS and MS/MS experiments were performed on a 7 Tesla Fourier transform-ion cyclotron resonance (FT-ICR) mass spectrometer equipped with an electrospray ionization (ESI) source, a hexapole ion cell floated with argon gas for collisional activation, a hollow dispenser cathode for electron emission and a continuous wave CO_2 laser for ion activation. ESI solutions were 1-2 µM RNA in 1:1 H_2O/CH_3OH with small amounts of organic bases as additives. ESI flow rate was 1.5 µl/min.

Results

In this study we combine EDD with infrared laser activation to disrupt residual secondary structure and to obtain new insights into the fragmentation mechanism. We found that infrared laser activation facilitates fragmentation of the radical ions without affecting the dissociation pathway. Our data also suggest that precursor ion charge ('Coulombic activation') is critical for electron detachment dissociation and therefore high sequence coverage.

By optimizing our EDD experiments accordingly we were able to achieve almost full sequence coverage from d/w ions for 76nt $tRNA^{Phe}$ from E. coli.

Conclusions

Activated ion EDD is suitable for top-down MS of RNA of up to 76 nt and provides data complementary to those from other dissociation methods such as CAD or IRMPD.

101 Chitosan-HIV 1 Tat Peptide conjugate: synergism with thiomer in enhanced gene transfection efficiency

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In this study nanoparticles were created with plasmid DNA (pDNA) and modified chitosans namely chitosan–thioglycolic acid (TGA) conjugate and chitosan–HIV-1 Tat peptide conjugate and evaluated for their efficacy as gene delivery agents. The transfection efficiencies of the complexes were assessed by the use of the green fluorescent protein (GFP) as reporter. HEK293 cells were incubated for 24 h with the nanoparticle samples and the GFP positive cells were scored by fluorescence microscopy. The transfection efficiency of the nanoparticles prepared by the combination of chitosan–TGA with chitosan–HIV-1 Tat peptide was relatively higher than that of the nanoparticles generated by either chitosan-TGA or the combination of unmodified chitosan with chitosan–HIV-1 Tat peptide. The results demonstrated a synergistic effect of chitosan-HIV-1 Tat and chitosan-TGA on transfection of the pDNA into the cells. Moreover, none of the nanoparticles tested were severely cytotoxic. Accordingly, this strategy might result in a promising carrier for delivery of genes.

Key words: HIV-1 Tat peptide, chitosan, synergism, gene delivery

102 An Animal Model for C-terminal Deregulation of Cav1.3 Channels

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Background: The C-terminal modulatory mechanism (CTM) is a strong determinant of channel gating properties within the α1-subunit of voltage gated $Ca_V1.3$ L-type Ca^{2+} channels (LTCCs). The CTM involves an intramolecular protein interaction between two putative α-helices (PCRD, DCRD) within the long C-terminal tail of the long splice variant $Ca_V1.3_L$. In a short splice variant ($Ca_V1.3_{42A}$) CTM is absent which leads to changes in channel gating: activation occurs at a more negative voltage range and Ca^{2+}-dependent inactivation is faster.

Methods: We quantified $Ca_V1.3$ splice variants by qPCR analysis and transcript scanning, using different mouse tissues. To assess the physiological relevance of CTM, we generated a $CTM^{-/-}$ mouse with standard techniques.

Results: We showed that $Ca_V1.3_{42A}$ RNA was less abundant in most mouse brain regions than the $Ca_V1.3_L$ (containing exon 49). The highest relative abundance was found in substantia nigra (SN) and ventral tegmental area (VTA). Quantitative PCR revealed that exon 49 containing transcripts were less abundant than exon 42, suggesting additional alternative splicing downstream of exon 42. We identified variant $Ca_V1.3_{43S}$, which comprises a shortened exon 43. Because of the absence of the DCRD, it functionally behaves as a short splice variant, despite the presence of the PCRD. We found that exon 43S-containing transcripts account for 40% of all exon 43 transcripts in mouse brain and conclude therefore that $Ca_V1.3_{43S}$ is the major short splice variant known so far. The generation of a $CTM^{-/-}$ mouse strain, in which CTM function is disrupted by an HA-tag, gave already rise to viable heterozygous offspring.

Conclusion: The $CTM^{-/-}$ model will allow to study the physiological role of CTM function *in vivo*. Furthermore, the HA-tagged α1-subunit will provide a tool to specifically determine the expression of $Ca_V1.3_L$ channels with anti-HA antibodies in mouse tissues.

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