Program overview

Wednesday, June 10th 2015

Oral Session 1 “Vascular Biology, Cardiology & Pulmonology; Endocrinology & Metabolism”, 9.30 - 10.30

Oral Session 2 “Immunology”, 12.30 - 13.45

Oral Session 3 “Molecular Biology; Molecular Pharmacology; Public Health”, 13.45 - 14.30

Poster Session “Endocrinology & Metabolism”, 15.00 - 17.00

Poster Session “Immunology”, 15.00 - 17.00

Poster Session “Molecular Biology”, 15.00 - 17.00

Poster Session “Molecular Pharmacology”, 15.00 - 17.00

Poster Session “Public Health”, 15.00 - 17.00

Thursday, June 11th 2015

Oral Session 4 “Cancer Research”, 10.00 - 11.00

Poster Session “Vascular Biology, Cardiology & Pulmonology”, 13.00 - 15.00

Poster Session “Cancer Research”, 13.00 - 15.00

Poster Session “Neurosciences & Psychiatry”, 13.00 - 15.00

Poster Session “Bones, Joints & Teeth”, 13.00 - 15.00

Poster Session “Medical Physics, Radiology, Biomedical Engineering & Informatics”, 13.00 - 15.00

Oral Session 5 “Neurosciences & Psychiatry; Bones, Joints & Teeth; Medical Physics, Radiology, Biomedical Engineering & Informatics”, 15.00 - 16.00
PLATELET PI3K IN ACUTE LUNG INJURY

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BACKGROUND: Acute lung injury (ALI) is a life threatening disease characterized by a damaged alveolar endothelium and epithelium, loss of the capillary-alveolar barrier, edema formation and infiltration of inflammatory cells, such as neutrophils. Neutrophil extravasation is mainly mediated by inflammatory cytokines, but recently it was shown that also platelets conduct neutrophil migration to sites of injury. One important kinase transmitting platelet activation is the phosphoinositide 3-kinase (PI3K). The aim of this project is to elucidate the influence of platelet PI3K on ALI.

METHODS: To study this task we bred mice with a megakaryocyte/platelet specific gene deletion of p85α one of the regulatory subunits of the PI3K. First, we examined the effects of p85α deficiency on platelet activation in vitro by measuring surface activation markers via flow cytometry and their potential to aggregate via light transmission aggregometry. Further, we treated mice intra-tracheally with hydrochloric acid (HCl) and analyzed leukocyte influx into the lung using flow cytometry and cytokine release by ELISA.

RESULTS: We found that platelets lacking p85α have a reduced surface expression of CD62P and CD40L compared to wild-type platelets in response to ADP (P2Y₁₂ agonist) and AY-NH₂ (PAR4 agonist). Additionally, the p85α knockout provokes a diminished aggregation after thrombin activation. Challenging knockout mice and wild-type littermates with HCl intra-tracheally, we observed attenuated leukocyte accumulation in the bronchoalveolar space and decreased cytokine release of interleukin-12 (IL-12) in the platelet p85α deficient mice.

CONCLUSIONS: These results indicate that PI3K is important to conduct platelet activation upon stimulation with various agonists. Lack of platelet PI3K reduces platelet activation and therefore likely diminishes leukocyte extravasation, leading to ameliorated symptoms in the murine model of ALI.

THE INFLUENCE OF S-NO-HSA ON CORONARY ARTERY ENDOTHELIUM IN ISOLATED MOUSE HEARTS UNDER NORMAL CONDITIONS AND IN REACTIVE HYPERAEMIA

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Background: S-NO-HSA has proven its positive effects in ischemia/reperfusion models to preserve endothelial and cardiac function. This study intends to investigate the vasodilatory potency on coronary arteries. Drug induced vasodilation is a promising therapeutic option for patients with cardiovascular disease. Additionally, the effects of S-NO-HSA are investigated after a short period of ischemia that provokes reactive hyperaemia, a phenomenon that could be modulated using S-NO-HSA. Materials and Methods: Hearts of male OF-1 mice are crystalloid perfused in a Langendorff-heart. After randomization, the experiment starts with an adapting-period of 15’ and measuring of baseline values. Afterwards, drug administration lasts for 10’ followed by 20’ of haemodynamic measurements. S-NO-HSA is tested during solely Langendorff perfusion (0,5µmol/kg/h, n=10; 5µmol/kg/h, n=3) to evaluate the extent of vasodilation. In the second part, after 5 minutes of drug administration, hearts undergo a 2 minutes period of global ischemia to provoke reactive hyperaemia (RH). Either S-NO-HSA (0,5µmol/kg/h+RH, n=10; 5µmol/kg/h+RH, n=7) or human serum albumin (control: n=5 and n=5) are administrated. Coronary flow (CF) and heart rate (HR) are monitored under constant afterload. Data are presented as mean±SEM compared to baseline (recovery in %). Results: HR remained stable in all groups and showed no significant changes between groups. CF recovery was increased in the 5µmol/kg/h S-NO-HSA group compared to 0,5µmol/kg/h S-NO-HSA (144,71% vs. 85,86%, p=0,011). Upon reperfusion, there is a trend of reducing RH with 0,5µmol/kg/h S-NO-HSA compared to its control (25,94% vs. 74,04%, p=0,076). There are no significant changes of 5µmol/kg/h S-NO-HSA to its control in RH. Conclusion: S-NO-HSA is able to dilate coronary arteries in a concentration of 5µmol/kg/h. 0,5µmol/kg/h S-NO-HSA is likely to decrease the extent of reactive hyperaemia provoked with 2´ global cardiac ischemia.

3 INTRARENAL ANGIOTENSIN II – ACE-INDEPENDENT PATHWAYS IN MOUSE AND MAN


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INTRODUCTION: The Renin-Angiotensin System (RAS) is involved in tissue remodeling and main regulator of blood pressure, fluid- and electrolyte-balance. In the kidney, multiple RAS enzymes have been described in animal models of disease and health, yet data on human RAS physiology is still lacking. AIMS: By comparing Angiotensin (Ang) II formation in healthy human kidneys from patients without or with RAS blockade (Ang-converting enzyme inhibitors (ACEi) or Ang receptor blockers (ARB)), we investigate RAS blockade-associated differences on intrarenal Ang II formation. Further, human kidneys are compared against mouse kidneys, to identify species-specific differences of Ang II formation. METHODS: Kidney biopsies of human living donors - treated with ACEi, ARB or no RAS blockade at all - and of C57BL/6 mice were snap frozen and later homogenized in physiological buffer at °C. Ang I was added to the homogenate and incubated at 37°C for one hour without (control) and with ACEi (lisinopril) and/or chymase-inhibitor (chymostatin). Generated Angs were isolated using C18 columns and quantified by LC-MS/MS. RESULTS: In mouse kidneys Ang II formation was completely blocked by exogenous ACEi, whereas in
patients either receiving no RAS blockade or ARB-therapy Ang II formation was blocked by 95% and in patients with ACEi-therapy Ang II formation was blocked by 69% (relative to control incubation). Combined blockade of ACE and Chymase abolished Ang II formation in all samples.

CONCLUSION: Here we show that subjects receiving ACEi-therapy exhibit significant ACE-independent Ang II formation in kidney tissue. These results uncover the intrarenal effects of ACEi in humans and might have repercussions for current and future treatment strategies involving RAS blockers.

4 POLARIZATION OF ADIPOSE TISSUE AND BONE-MARROW-DERIVED MACROPHAGES IS ALTERED IN OSTEOPONTIN KNOCK-OUT MICE

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Macrophages are the main drivers of obesity-induced adipose tissue (AT) inflammation that leads to major health impairments. In lean AT, anti-inflammatory M2 macrophages dominate, while in obesity the phenotype of macrophages changes into a pro-inflammatory M1 type by yet unknown mechanisms. A protein highly expressed in AT of obese is osteopontin (OPN). OPN is an extracellular matrix protein with chemokine and cytokine functions that is secreted by macrophages and was shown to contribute to AT inflammation and insulin resistance. We hypothesized an impact of OPN on the phenotype of macrophages. OPN knockout and wild type C57BL/6 (WT) mice were set on a high fat diet for 0, 4, 8 and 12 weeks and total gonadal AT as well as AT macrophages (ATM) were isolated and analyzed by flow cytometry and for gene expression. Bone marrow derived macrophages (BMDM) of OPN-/- and WT mice were polarized with OPN for two days and surface markers were analyzed. Although OPN knockout ATM surprisingly tended to express more of the ATM M1 surface marker CD11c they did not up-regulate inflammatory cytokine (Il1b, Il6) gene expression and Tnf was even lower expressed in OPN knockout ATM. Conversely, the M2 markers arginase and mannose receptor (CD206) tended to be less expressed on mRNA and cell surface level, whereas Fizz1 and CD36 expression did not differ between knockouts and WT. On the other hand, OPN-deficient BMDMs expressed more CD206 and less M1 surface marker CD80 and addition of exogenous OPN resulted in an up-regulation of CD80. The surface phenotype of ATM in OPN deficient mice tended to be polarized rather to M1 as compared to WT while OPN-deficient BMDMs were polarized to an M2 surface phenotype. Hence, the detailed role of OPN in obesity-induced AT inflammation remains to be investigated. Supported by the Federal Ministry of Economy, Family and Youth and the National Foundation for Research, Technology and Development (to T.M.S.).
5 MTORC2 REGULATES MACROPHAGE POLARIZATION AND THE CELLULAR ENERGY METABOLISM

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Macrophages are innate immune cells and pivotal for the regulation of immune and metabolic responses. A dysregulation of immune or metabolic processes contributes to or drives many chronic diseases such as obesity, cancer, or autoimmunity. The mammalian target of rapamycin complex 2 (mTORC2) is an important kinase that regulates many basic cellular and metabolic processes; however, its function in macrophages is largely ill-defined. The aim of our project is to understand the role of mTORC2 for macrophage polarization and the associated implications on cellular and whole body metabolism. In vitro, we show that deletion of the mTORC2 component Rictor (rapamycin-insensitive companion of mTOR) in macrophages leads to a stronger inflammatory M1 phenotype and reduced M2 polarization potential suggesting a possible function of mTORC2 in macrophages in the regulation of inflammatory responses. However, this inflammatory phenotype does not seem to have an effect on whole body glucose metabolism in vivo, as glucose- and insulin-tolerance tests do not yield significant differences between control mice and mice with a macrophage specific knockout of Rictor. Interestingly, less macrophages accumulate in adipose tissue of macrophage specific Rictor-KO mice on high fat diet compared to control mice. In vitro, we find that the knockout of Rictor diminishes cell proliferation of macrophages in the presence of M-CSF, but does not influence apoptosis. Moreover, the migratory capacity of macrophages is higher if mTORC2 is intact. Molecularly, glucose influx and mitochondrial potential are reduced in Rictor-deficient macrophages. In conclusion, our results point to a role of mTORC2 in the regulation of macrophage polarization, cell cycle progression and migration that might be important in the onset of severe inflammatory diseases and suggest inhibition of mTORC2 as a possible therapeutic approach.

6 TREGS INDUCE MIXED CHIMERISM BY SUPPRESSING COSTIMULATION BLOCKADE RESISTANT NK CELLS

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The adoptive transfer of in vitro activated regulatory T cells (Tregs) obviates the need for cytoreductive conditioning in a costimulation blockade based (BL/6 to Balb/c) bone marrow transplantation (BMT) model and induces long lasting tolerance to skin and heart allografts. We hypothesized that Tregs would enable bone marrow engraftment by suppressing NK cells. To test our hypothesis we replaced Treg therapy by NK cell depleting antibodies or used F1 mice (offspring of a cross between Balb/c females and BL/6 males) as bone marrow donors as their cells do not activate recipient NK cells. C57BL/6 mice
received 20x10^ unseparated Balb/c or F1 bone marrow cells under costimulation blockade (anti-CD154mAb, CTLA4-Ig) and a short course of rapamycin. Mice transplanted with Balb/c bone marrow were additionally treated with NK cell depleting antibodies (anit-NK1.1) or co-injected with 1x106 in vitro activated Tregs. All BMT recipients received skin grafts and selected recipients also cardiac grafts. Costimulation blockade or rapamycin had no impact on NK cell mediated rejection implying that Tregs are primarily responsible for this purpose. NK cell depletion at the time of BMT efficiently replaced adoptive Treg transfer as all mice developed persistent mixed chimerism (7/7). Nevertheless, more than half of NK cell depleted recipients chronically rejected their skin grafts (4/7). In contrast, mice grafted with CB6F1 bone marrow developed stable mixed chimerism and retained their allografts indefinitely without any histological signs of rejection (5/5) probably due to the unresponsiveness of recipient NK cells to alloantigens. In line with this, the absence of chronic rejection in Treg treated mice correlated with the adaption of recipient NK cell receptors to donor antigens. Therefore we conclude that adoptively transferred Tregs prevent bone marrow rejection by suppressing costimulation blockade resistant NK cells and promote the accomodation of NK cells to donor cells.

7 A POSSIBLE ROLE OF NEUTROPHILS IN IGE MEDIATED ALLERGY

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Background: Although large numbers of neutrophils are present in late-phase reactions, their role in allergic disorders is not well understood. These professional phagocytes can be activated to express MHC class II molecules by stimulation with certain cytokines, chemokines and bacterial factors, such as GM-CSF, TNF-α, IL-8, IFN-γ and LPS. Some studies have even shown that murine neutrophils are able to process and present antigens to CD4+ T-cells. Aim: To assess whether human neutrophils act as antigen-presenting (APC) cells for allergen-specific T-cells. Methods: Neutrophils isolated from peripheral blood from allergic patients were analyzed for the expression of MHC class II and co-stimulatory molecules under different culture conditions by flow cytometry. Surface binding, internalization and intracellular degradation of fluorescence-labelled Bet v 1, the major birch pollen allergen, by neutrophils were compared with monocytes. Endolysosomal proteases were isolated from neutrophils and monocytes. Finally, neutrophils and monocytes were used as APC for Bet v 1-specific T-cell lines and clones generated from birch-pollen allergic donors. Results: The mixture of IL-3, GM-CSF and IFN-γ enhanced the expression of HLA class II and CD80 on neutrophils. Neutrophils effectively internalized and degraded Bet v 1 faster then monocytes. Neutrophils pulsed with Bet v 1 induced proliferation in Bet v 1 specific T cells specific for different epitopes. Conclusions: Our data provide evidence that neutrophils may serve as antigen presenting cells for allergen specific T-cells in certain cytokine milieu, as it is found in the late phase reaction. Supported by the Austrian Science Funds, project W1248 and SFB F4610.

8 A NOVEL TSC2-MTORC1-CDK4 AXIS CONTROLS TISSUE HOMEOSTASIS BY REGULATING POLARIZATION, PROLIFERATION, AND METABOLISM IN MACROPHAGES
Maintenance of tissue homeostasis requires a tight control of the in situ-proliferative capacity of tissue-resident M2-like macrophages. However, the signaling pathways that regulate quiescence versus proliferation in tissue macrophages are unknown. Here we investigated a potential role of tuberous sclerosis 2 (Tsc2), the key negative regulator of mammalian target of rapamycin complex 1 (mTORC1), for this process. Deletion of Tsc2 in myeloid cells broke quiescence and strongly induced macrophage proliferation and granuloma formation in vivo in an mTORC1-dependent manner. Intriguingly, Tsc2-mTORC1 directly controlled M-CSF-stimulated cell cycle progression by inducing the expression of cyclin-dependent kinase 4 (CDK4), while repressing inflammatory NF-κB signaling. Tsc2-deficient macrophages showed constitutive CDK4 expression and enhanced M2-like polarization that was accompanied by a reconfiguration of the cellular metabolism towards increased aerobic glycolysis and mitochondrial metabolism. Inhibition of mTORC1, CDK4, and hexokinase abrogated cell cycle progression, indicating an intrinsic need of glucose flux and CDK4 for M-CSF-dependent M2-like macrophage proliferation. Strikingly, we found that CDK4 and glucose flux directly contributed to the M2-like polarization potential of Tsc2-deficient macrophages. In conclusion, we demonstrate that activation of an mTORC1-CDK4 axis stimulates macrophage proliferation, M2 polarization and metabolic reconfiguration, promoting granulomatous disease in vivo. Generally, our data shows for the first time that cell cycle regulation in mammals is controlled by the quantitative regulation of a CDK. The precise molecular elucidation of how macrophages dynamically regulate proliferation versus quiescence will have fundamental implications for the understanding of tissue homeostasis and immunity, which might lead to novel therapeutic targets for granulomatous diseases.

9 LIVE FAST – DIE YOUNG: UROKINASE RECEPTOR EXPRESSING T CELLS ARE ADHERENT, HYPER-REACTIVE AND UNDERGO ACTIVATION INDUCED CELL DEATH

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T cell activation and migration of T cells are two central processes in adaptive immunity. We are interested in the urokinase receptor (uPAR, CD87), which equips T cells with features important in both of them. uPAR is widely recognized as a mediator of fibrinolysis, cell adhesion, migration and extracellular matrix (ECM) degradation. It is hardly expressed on the surface of resting human peripheral T lymphocytes, however, it appears upon activation (Nykjaer et al 1994). Furthermore, there is evidence that it is important in T lymphocyte migration to sites of infection (Gyetko et al 2001) and also to tumors (Edwards et al 2006). Yet, detailed molecular signaling mechanisms in T cells as well as uPAR’s role in aspects apart from migration have so far not been investigated. We therefore examine uPAR’s function in T cell activation and further scrutinize its role in T cell migration in more detail. We found that T cells overexpressing uPAR change their phenotype from suspension to adherent cells because of strong adhesion to the ECM component vitronectin. In addition, these cells display an elevated response to T cell receptor stimulation as measured by calcium mobilization and IL-2 production. However, this hyper-reactivity of uPAR overexpressing T cells ultimately leads to activation-induced cell death, which can be inhibited by mAbs that block the interaction of uPAR with its natural ligands urokinase and vitronectin. Thereby the lifespan of reactive T cells destined to undergo apoptosis could be prolonged, which might be of use in therapeutic interventions.
HEPATOCYTE SPECIFIC EXPRESSION OF A DOMINANT STABLE FORM OF B-CATENIN RESULTS IN CHOLESTATIC LIVER DISEASE


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Background: The Wnt/b-catenin signaling pathway is an ancient, evolutionary preserved systems, which plays a crucial role in embryonic development, tissue homeostasis, wound healing and malignant transformation in a variety of different organs, including the liver. To investigate the role of continuous β-catenin signalling in liver biology, we generated mice, which lack exon 3 of the ctnnb1 gene specifically in hepatocytes. Methods: Mice with a loxP flanked exon 3 of the ctnnb1 gene were crossed to albumin-Cre mice to obtain mice with hepatocyte specific expression of a dominant stable form of β-catenin (catn ex3Δ hep mice). Results: Successful removal of exon 3 of the ctnnb1 gene was confirmed by immunoblotting using whole liver tissue. Mice were born at the expected Mendelian ratio (Cre positive: 53.1%). Expression of a degradation resistant form of β-catenin in hepatocytes resulted in 100% mortality as early as at 31 days of age (median survival 26 days). Livers of Ctnnb1CA hep mice were smaller and their body weight was significantly reduced. Serum ALT and AST levels were significantly increased in Ctnnb1CA hep mice and serum bile acid levels were ~25 fold increased in Ctnnb1CA hep mice (243.8 vs. 10.5 µmol/L; p<0001). Microscopically, livers of Ctnnb1CA hep mice displayed a disturbed liver architecture, paucity of central veins, mitotic figures, signs of extramedullary hematopoiesis and reactive cholangiocytes. Sirius red and desmin staining indicated a biliary type of fibrosis. Ctnnb1CA hep mice did not differ from Cre-negative controls with regards to expression of bsep as evaluated by qPCR. In contrast, expression of mRNA levels of compensatory bile acid transporters including abcb4, mdr1a and mrp2 were significantly increased in Ctnnb1CA hep mice. Consistent with increased expression of compensatory bile acid transporters at the apical lumen, Ctnnb1CA hep mice displayed significantly reduced mRNA levels of ntcp but significantly increased levels of mrp4 suggesting activation of a Constitutive Androstane Receptor (CAR)-mediated protection pathway leading to secondary detoxification of bile acids. Expression of cyp7a1 the rate-limiting step in bile acid synthesis was significantly increased in Ctnnb1CA hep mice. In
contrast, expression of cyp27, cyp8b1 and cyp2b10 was reduced by 4-, 20- and 200-fold in Ctnnb1CA hep mice compared to Cre-negative littermate controls. Conclusion: Expression of a dominant stable form of β-catenin in hepatocytes results in severe cholestasis, biliary type fibrosis and premature death.

11 DISCOVERY AND CHARACTERIZATION OF NOVEL OXYTOCIN-LIKE PEPTIDES: BEYOND CLASSICAL LIGANDS FOR HUMAN OXYTOCIN AND VASOPRESSIN RECEPTORS

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Oxytocin (OT) and arginine vasopressin (AVP) are neuropeptide hormones implicated in the regulation of crucial physiological functions such as uterine contraction and social behavior. In humans, they signal via four known G protein-coupled receptors OT, V1a, V1b and V2 receptors. Sequence homology of the ligands and structural similarity of the receptor subtypes pose several challenges for the design and development of selective agonists and antagonists. Certain invertebrates contain a closely related neuropeptide signalling system important for reproduction and learning. Our aim is to study the OT/AVP signalling system and to harness its molecular and evolutionary conservation for the characterization of novel peptide analogues with improved selectivity on human OT and AVP receptors. We utilized transcriptome sequencing to obtain peptide and receptor sequences of our model ant species Lasius. The cognate receptor sequence was validated by PCR and the specific interaction with its native ligand inotocin was confirmed by in vitro pharmacology. To improve the stability of the inotocin peptide in vivo, several peptides with D-amino acid substitutions were synthesized. Their affinity and efficacy for the human OT and AVP receptors were determined via displacement binding experiments and quantitative second messenger analysis. These studies demonstrate that the invertebrate ligand is able to selectively activate human AVP receptors. Moreover, D-amino acid replacement of specific residues yields a functional switch at the human vasopressin V1b and V1a receptors. Affinity vs. activity can be selectively modulated revealing a rational strategy for the design of selective antagonists with enhanced affinity to V1bR and V1aR compared to OT and V2 receptors. In summary, naturally-occurring peptides can be used as pharmacological tools to modulate activity and affinity on human receptors, with an evolutionary advantage over traditional combinatorial chemistry approaches.

12 DISORDERED EATING BEHAVIOURS AND RELATED RISK AND PROTECTIVE FACTORS: RESULTS FROM THE FIRST EPIDEMIOLOGICAL SURVEY IN AUSTRIA


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No representative epidemiological data about the prevalence of disordered eating behaviours is available for Austrian children and adolescents up to now. One aim of the MHAT (Mental Health in Austrian Teenagers)-Study is to investigate the prevalence of disordered eating behaviours as well as related risk and protective factors and quality of life in a representative sample. A large population sample (3610 adolescents, 55% girls) aged 10 to 18 years was recruited from Austrian schools. The SCOFF questionnaire was used to assess disordered eating behaviours, the Youth Self-Report and the KIDSCREEN to identify emotional and behavioral problems and quality of life. Social and demographic correlates, risk and protective factors were also assessed. 30.9% of girls and 14.6% of boys scored above the defined cut-off score of 2 in the SCOFF questionnaire. Prevalent symptoms were “food thoughts”, “losing control over food” and “body dissatisfaction”. Only “weight loss” was slightly more prevalent in boys. “Intentional vomiting” was less prevalent. Higher BMI was associated with more eating problems. Identified risk factors were “low socioeconomic status” (girls OR=2.1; boys OR=1.5), “stressful life events” (girls OR=1.5; boys OR=1.7), “experience of violence and abuse” (girls OR=1.8; boys OR=2.0), “absence of an adult attachment figure” (girls OR=2.0; boys OR=1.8) and “physical illness” (girls OR=1.5; boys OR=1.6). Further risk factors were “social support”, “absence of a biological parent” as well as “physical” or “mental illness within the family” for girls and “migration background” for boys. Eating problems were further associated with emotional and behavioural problems and low quality of life. In conclusion, our study indicate high prevalence of disordered eating behaviours for female and male adolescents (especially for girls and overweight adolescents), confirming other studies like the German KIGGS-Study. Analyses of risk factors give useful hints for preventive work.
21 SYSTEMIC METABOLIC DEFECTS CAUSED BY EPIDERMAL EGFR-DEFICIENCY

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The epidermal growth factor receptor (EGFR) is an important regulator of epidermal function and homeostasis. Epidermal deletion of EGFR leads to severely perturbed skin differentiation and causes reduced animal growth and lethality during the first three weeks of life. The molecular cause why animals lacking EGFR in the epidermis die soon after birth is still unclear. It is known that these animals develop a severe skin inflammation and a skin barrier defect; however both of these pathological features become apparent only after these mice already show reduced growth and weight gain. In order to better understand the growth defect observed in these mice, metabolic parameters have been started to be analyzed revealing that epidermal loss of EGFR-signaling results in severely perturbed glucose metabolism and insulin levels in the blood of affected animals. Importantly, gene expression of metabolic regulators in livers of mice lacking EGFR in the epidermis indicates that glucose metabolism is deregulated already a few days after birth. In addition, the “starvation-marker” FGF-21 is highly expressed in these animals. To test whether excessive feeding protects animals with defective epidermal EGFR signaling from death, these mice were crossed in a leptin-deficient background and found that this fully rescues the lethality. Furthermore, animal growth as well as glucose and insulin levels are improved in such animals, demonstrating that the lethality caused by epidermal EGFR-deficiency stems from impaired food metabolization. We are currently searching for molecular mediators of the metabolic dysfunction of mice lacking EGFR in the epidermis. These findings will provide new insights into the complex consequences of epidermal Egfr-deficiency, which might also be relevant for cancer patients treated with Egfr inhibitors. Furthermore, results obtained by this study will lead to a better understanding of the role of the skin in the regulation of systemic metabolism.

22 Suppression of plasma free fatty acids similarly reduces myocardial lipid content and left ventricular systolic function in type 2 diabetic patients and controls

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Background and aims: Type 2 diabetes (T2DM) is closely associated with the development of heart failure, in part due to impaired substrate metabolism and accumulation of myocardial lipids (MYCL). To better understand substrate metabolism of the diabetic heart, this study investigated the impact of an acute pharmacological inhibition of adipose tissue lipolysis leading to reduced availability of circulating
FFA on MYCL and heart function in T2DM. Materials and methods: 8 T2DM (Age: 56±11a; BMI: 28±3.5 kg/m2; HbA1c: 7.29±0.88%) were investigated on two study days in random order, following administration of Acipimox /Placebo and compared to previously assessed data in healthy controls. MYCL and heart function was measured by 1H-magnetic-resonance-spectroscopy and tomography at baseline, at 2 and at 6 hours. Results: Acipimox reduced MYCL by -39±41% as well as systolic heart function significantly (Ejection Fraction (EF): -13±8 and cardiac index: -16±15% compared to baseline) to the same extent than in healthy controls., FFA strongly correlated with changes in MYCL (ΔMYCLMR1vsMR3: r=0.707; p=0.002) and EF (ΔEFMR1vsMR3: r=0.651; p=0.006). Diastolic heart function remained unchanged. Conclusion: Inhibition of adipose tissue lipolysis is associated with a rapid depletion of MYCL-stores and reduced systolic heart function in T2DM. These changes were comparable to those previously found in insulin sensitive controls. MYCL thus likely serves as a readily available energy source to cope with short-time changes in FFA availability in the diabetic state.

23 IDENTIFICATION OF KEY MOLECULES FOR CARDIO-METABOLIC DISEASE

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Obesity is associated with the Metabolic Syndrome, a compilation of factors conferring a risk for cardiometabolic disease, i.e. atherosclerotic cardiovascular disease [CVD] and type 2 diabetes[T2D]. The objective of this study was to identify key molecules to be potentially targeted for prevention and treatment of CVD and T2D. In order to find upregulated and common proteins expressed in visceral adipose tissue[VAT] and atherosclerotic vascular wall from the aorta [AA], an insulin resistance/atherosclerosis mouse model established in our lab (Neuhofer, Wernly et al. 2014) was used. Wild type [WT] and LDLR knockout mice [LDLR-/-] were fed chow diet [CD], 0,15% of cholesterol enriched high-fat diet [HFC] or a sucrose-enriched HFC [HFSC] for 20 weeks. Tissues were collected from VAT and AA. RNA was isolated for Affymetrix arrays analysis to evaluate gene expression (GeneChip Mouse Exon 2.0 ST Array). Primary data was analyzed with Expression Console software (Affymetrix). Student’s t test scores (p-values) were calculated with Microsoft Excel (Microsoft Corp.) and the fold change [FC] of each gene was obtained comparing case and controls. The analysis of the VAT revealed: 11632 different expressed genes [DEG] in WT on HFC vs WT on CD and 11182 DEG in LDLR-/- on HFSC vs LDLR-/- on CD. In addition AA analysis showed: 1081 DEG in LDLR-/- on CD vs WT on CD, 2025 DEG in LDLR-/- on HFSC vs WT on CD and 2242 DEG in LDLR-/- on HFSC vs LDLR-/- on CD. New molecules that are implicated in both metabolic and cardiovascular disease will be sought considering inflammation as a key mechanism linking obesity, T2D, and CVD. This work is supported by the Federal Ministry of Economy, Family and Youth and the National Foundation for Research, Technology and Development (to T.M.S).

24 CORRELATION IN LATERALIZATION OF OOCYTE YIELD AND EMBRYO QUALITY WITH PATIENTS’ HANDEDNESS

Introduction: Different studies revealed that follicle and oocyte number is higher on the right side. Higher pregnancy rates from oocytes of the right ovary have been described. Ovarian cancer has a right side tendency while endometriomas appear more frequent on the left side. Material and methods Prospective multi-center cohort study on 950 patients between 2009 and 2014. Follicle count, number of oocytes, metaphase 2 oocytes, number of embryos, and top quality embryos were evaluated on the left vs. the right side. Patients with PCOS, ovarian endometriosis, hemiovariectomy and asthenozoospermia of the partner have been excluded. Patients undergoing IVF/ICSI treatment have been asked if they were right or left handed. This information has been noted and blinded. At oocyte retrieval oocytes have been separated according to which oocytes come from the left- and from the right ovary. Results 784 (82.5%) right-handers and 166 (17.5%) left-handers have been investigated. Right-handers had more follicles (4.63 right vs. 3.86 left, P<0.001), oocytes (3.89 right vs. 3.00 left, P<0.001), metaphase 2 oocytes (3.05 right vs 2.17 left, P<0.001), embryos (2.45 right vs 1.72 left, P<0.001) and top quality embryos (0.80 right vs. 0.46 left, P<0.001) on the right side while left-handers had more follicles (3.31 right vs 4.25 left, P=0.001), oocytes (2.32 right vs. 4.01 left, P<0.001), metaphase 2 oocytes (1.92 right vs. 3.32 left, P<0.001), embryos (1.58 right vs. 2.72 left, P<0.001) and top quality embryos (0.57 right vs. 1.04 left, P=0.003) on the left side. Conclusio The present study suggests that laterality is not only taking place in the central nervous system but also at the ovarian level according to central lateralization.

OSTEOPONTIN INDUCES THE NFKB PATHWAY ACTIVATION IN VITAMIN D3 - DIFFERENTIATED U937 CELLS

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Introduction Osteopontin (OPN) is a cytokine well-expressed in activated macrophages and found to be highly upregulated in obese adipose tissue (AT). It is known that obesity is associated with chronic low-grade inflammation, which is characterized by infiltration of AT with activated macrophages. Macrophages are the main producers of cytokines involved in the onset of insulin resistance and, consequently, type 2 diabetes. Via binding to various surface receptors, cytokines such as OPN act as mediators of inflammatory processes. Therefore, adipose tissue macrophages (ATM) together with inflammatory molecules such as OPN, appear indeed important targets in prevention and treatment of type 2 diabetes. Materials and methods CD44 and variants as well as a number of integrins have been shown to be receptors for OPN. In order to elucidate whether OPN’s effect on macrophages is mediated via integrins, we aimed at establishing an OPN-induced integrin signaling model system. U937 cells stably transfected with GFP under control of a NFκB-driven promoter (U937-GFP) were differentiated to macrophages by vitamin D and tested for reactivity to OPN and integrin abundance by flow cytometry.
Results Several OPN preparations showed a good effect on the activation of U937-GFP cells. Reactivity to OPN was in some cases 20 fold enhanced in cells differentiated with Vitamin D against non-treated counterparts. Integrin chains α4, α5 and β1 were found to be highly expressed in U937-GFP cells.

Conclusion In conclusion, our findings emphasize a putative role of integrins in OPN action in macrophages. Which may help to develop OPN-inhibitory strategies for prevention and treatment of type 2 diabetes in obese individuals. This work is supported by the Federal Ministry of Economy, Family and Youth and the National Foundation for Research, Technology and Development (to T.M.S.).

26 EVALUATION OF THE FUNCTIONALITY OF THE HUMANIZED OSTEOPONTIN MOUSE AS A DIET-INDUCED OBESITY MODEL

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Obesity is associated with an inflammatory state, thereby elevating pro-inflammatory cytokines and macrophages in the adipose tissue. Adipose tissue inflammation is the key factor causing insulin resistance which underlays type 2 diabetes. Additionally, obesity results in a tremendous economic and medical burden. Therefore, it is relevant to evolve new strategies for the treatment of obesity-associated diseases, like type 2 diabetes or atherosclerosis. Osteopontin (OPN), a well-known inflammatory cytokine, plays a fundamental role in adipose tissue inflammation and insulin resistance. During inflammation, OPN is proteolytically cleaved by thrombin or matrix metalloproteinases that increases its activity through exposure of otherwise cryptic regions. Therefore, OPN provides a new interesting target for treatment of obesity-associated diseases. In order to specifically target certain regions of the OPN molecule, an immunotherapeutic approach is to be developed to prevent cardio-metabolic disease. Based on this background, we wanted to evaluate the functionality of a humanized OPN mouse model as a diet-induced obesity model. Humanized OPN (hOPN) and C57BL/6Jrj mice were set on either a high-fat diet (HFD) or a low-fat diet (LFD) for 22 weeks. Weight gain, inflammatory and metabolic markers were assessed by using quantitative real-time PCR or enzyme-linked immunosorbent assay. We could show that hOPN mice generate diet-induced obesity when fed a HFD. Moreover, fasting glucose levels as well as inflammatory and metabolic markers were significantly upregulated in hOPN mice on HFD. Our data strongly supports the idea that our hOPN mouse can be applied as a diet-induced obesity model to study differences in human and murine OPN. Furthermore, the hOPN mouse model is necessary to test OPN peptides for immunization. This work is supported by the Federal Ministry of Economy, Family and Youth and the National Foundation for Research, Technology and Development (to T.M.S.).

27 IMPEDING CELLULAR ADHESION IN VITRO BY TARGETING THE CRYPTIC INTEGRIN-BINDING MOTIF SVVYG(LR) OF MATRIX METALLOPROTEINASE- OR THROMBIN-CLEAVED OSTEOPONTIN WITH ANTIBODIES OR ANTISERA

Osteopontin (OPN) is a matrix associated protein involved in many inflammatory diseases. It is highly expressed cytokine during adipose tissue inflammation, atherosclerosis, rheumatoid arthritis, and cancer. Immune cells adhere, and migrate to OPN through binding to a canonical RGD integrin binding motif. Furthermore, matrix metalloprotease (MMP) or thrombin exposure cleaves OPN and reveals a cryptic SVVYG(LR) integrin binding motif, which exaggerates cellular adhesion. We targeted this neoepitope with monoclonal antibodies or antisera induced by peptide immunization of mice to inhibit cellular adhesion in vitro. Hence, we performed adhesion assays with fluorescent-labeled HEK 293 cells. Recombinant full length, N-terminal thrombin-, or MMP-cleaved human OPN was immobilized on V-well microtiter plates and adherence-impeding properties of our antibodies or antisera were investigated. We could show that HEK 293 did adhere significantly stronger to the truncated OPN forms than to full length OPN. Of note, the tested antibodies impeded the adhesion specifically either to the MMP cleaved OPN or to both truncated OPN forms, whereas antisera showed specificity solely against the immunized OPN fragment. In conclusion, specific targeting of OPN’s integrin binding motifs with antibodies or antisera impedes cellular adhesion towards cleaved OPN forms in vitro. These results may pose a first step towards developing immunotherapeutic treatments against inflammatory diseases that involve cleaved OPN fragments. Supported by the Federal Ministry of Economy, Family and Youth and the National Foundation for Research, Technology and Development (to T.M.S.).

28 THE ROLE OF ADIPONUTRIN (PNPLA3) IN HEPATIC STELLATE CELL ACTIVATION

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Background: Adiponutrin (PNPLA3) is a nutritionally regulated protein that has triglyceride hydrolase and/or lysophosphatidic acid acyltransferase activities in vitro. A genetic variant of PNPLA3, the I148M isoform, is strongly associated with NAFLD and progression towards fibrosis. Hepatic stellate cells (HSCs) are the key cells in hepatic fibrosis, but little is known about the role of PNPLA3 and fatty acids (FA) metabolism as potential key players in hepatic lipotoxicity and fibrogenesis.Aims: Explore the metabolic role of PNPLA3 in HSCs activation and investigate its impact on fibrogenesis.Methods: LX2 (HSC cell lines) were challenged with various fatty acids. We performed western blot analysis, real-time PCR, migration assays and Oil red O staining.Results:Challenge of LX2 cells with oleic acid (OA) results in a significant increase of PNPLA3 at both protein and mRNA level (maximally 3-fold, after 50 micromolar oleic acid) which correlates with increased cell migration, induction of Collagen1α1 (mRNA), α-smooth muscle actin (α-SMA) and microtubule-associated proteins 1 light chain 3 (LC3A/B) proteins as profibrogenic and autophagy markers, respectively. Oil red O staining revealed accumulation of lipid droplets (LDs) in presence of OA. Silencing PNPLA3 by 85% with specific siRNA resulted in a 30% decrease of alpha-SMA, while oil red O staining showed an increased accumulation of lipid droplets (LDs) by 40% compared to
scrambled siRNA controls. Treatment with oleic acid along with TGF-beta led to a significant decrease in LDs (p<0.01) and an increase in both PNPLA3 and alpha-SMA protein amount, while siRNA knockdown of PNPLA3 in cells subsequently stimulated with TGF-beta showed a significant attenuation (p<0.01) in alpha-SMA protein levels. Conclusion: Reduction of PNPLA3 correlates to a decreased expression of fibrotic markers and increased accumulation of LDs in HSC suggesting that PNPLA3 acts as a compensatory lipase in HSC facilitating release of excess FA.

29 EPIGENOME-WIDE SCREEN FOR ALPHA TO BETA CELL TRANSDIFFERENTIATION

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Within the pancreatic islets of Langerhans, α- and β-cells tightly maintain glucose homeostasis via the controlled release of the hormones glucagon and insulin. During Type I Diabetes (T1D), this tight interaction is deregulated with the targeted destruction of β-cells, resulting in severe hyperglycemia. The loss of secreted insulin and β-cell mass underscores the need for a new insulin source in T1D patients. The close developmental relationship between α- and β-cells, evident via their common Ngn+ progenitor, make α-cells an effective candidate. What’s more, previous studies have revealed the ability of α-cells to adopt functional characteristics of β-cells upon epigenetic manipulation. Hence, further epigenetic manipulation of this α-β-cell relationship could aid in understanding their transdifferentiation capacities and allow for potential β-cell mass replenishment. Consequently, we set out to uncover new proteins that induce β-cell properties in α-cells. We constructed an epigenetically focused RNAi library, encompassing approximately 300 gene knockdowns. We applied this library to the murine, immortalized α-cell line, αTC1 cells, via viral delivery of short hairpin RNA sequences. We screened the cells for changes in their transcription profile, with a particular focus on increased insulin (Ins2) expression. One of the hits was a gene involved in post-translational modifications. We are now in the process of validating it as an effective α-to-β cell transdifferentiation factor, as well as running proteomics experiments to identify mode of action of the protein and functionally characterize the resulting β-like cells. Initial RNAseq data revealed an upregulation in β-cell markers, including Iapp, gck, Pax4 and Ins2, upon knockdown in αTC1 cells. Overall, α-cell-targeted loss of this gene might provide us with a new insulin cell source.

30 EFFECTS OF INORGANIC PHOSPHATE AND FGF23 ON C2C12 MYOBLAST CELLS

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Objectives: Dysregulation of systemic phosphate homeostasis is associated with impairment of musculoskeletal tissue function. Many factors such as calcium levels and dysegulated endocrine mechanisms are also thought to contribute. Inorganic phosphate and FGF23 have been shown to act via similar signaling pathways in several cell types but we are not aware of any detailed investigations into their effect on the differentiation and viability of skeletal muscle cells. We therefore investigated their
effect on skeletal muscle cells in a murine in vitro model. Methods: C2C12 muscle progenitor cells were differentiated under single and combined treatments with inorganic phosphate and/or FGF23 and Klotho. Expression of differentiation markers (myogenin, MyHC, MyoD, Myf5) were analyzed by RT-PCR. Proliferation rate was analyzed by measurement of BrdU incorporation. Metabolic activity was examined by EZ4U assays. Results: Phosphate treatments inhibited the expression of differentiation markers in C2C12 cells in a dose-dependent manner. The altered expression profile was associated with increased proliferation rates and metabolic activity. FGF23/ Klotho treatments did not alter gene expression of C2C12 cells or change the effects observed under phosphate treatment. Conclusion: High phosphate loads directly inhibited muscle cell differentiation in a C2C12 model system. FGF23/ Klotho treatments did not influence these effects. Knowledge of the distinct effects of phosphate could help us to optimize treatment of hypophosphatemia and ultimately to prevent musculoskeletal diseases.

31 EMODIN, A COMPOUND WITH PUTATIVE ANTIDIABETIC POTENTIAL, DETERIORATES GLUCOSE TOLERANCE IN RODENTS

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32 REDUCED T CELL INFILTRATION INTO ADIPOSE TISSUE IN OSTEOPONTIN KNOCKOUT MICE AND INHIBITION OF T CELL ADHESION TO OSTEOPONTIN

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Obesity is associated with a chronic low-grade inflammatory response, originating in adipose tissue and interfering with the insulin signaling cascade, thereby promoting insulin resistance and type 2 diabetes development. Osteopontin (OPN) is an extracellular matrix protein and inflammatory cytokine, the expression of which is strongly upregulated in obesity. OPN is implicated in the polarization and migration of T cells. In the onset of adipose tissue inflammation a shift of the predominant T cells from an anti-inflammatory to pro-inflammatory phenotype occurs. I, therefore, hypothesized that OPN promotes adipose tissue inflammation also by affecting T cells. In this PhD project, the role of OPN in the infiltration of T cells into adipose tissue and the possibility to interfere with T cell adhesion to OPN is to
be elucidated. To investigate the former, we put wt and OPN KO mice for 4 and 8 weeks on high fat diet. After 4 weeks, less T cells were present in the adipose tissue of OPN KO mice versus wt mice as assessed by FACS analysis. This implies that OPN plays an important role in the migration of T cells into adipose tissue. To block the binding between T cells and OPN in vitro, we analyzed the adhesion of Jurkat T cells and primary human T cells to OPN. In this assay the adhesion of fluorescently labelled T cells to recombinant OPN is assessed. In-house developed monoclonal antibodies against OPN peptides and mouse sera raised against injected OPN-peptides significantly interfered with T cell adhesion to OPN. Importantly, we could specifically inhibit the binding of T cells to cleaved forms of OPN which are present at sites of inflammation. In conclusion, targeting OPN by specific monoclonal antibodies to interfere with T cell adhesion and hence migration could be a novel therapeutic approach to reduce adipose tissue inflammation thereby preventing insulin resistance and type 2 diabetes.

33 ABDOMINAL OBESITY EVALUATED BY WAIST-TO-HEIGHT-RATIO INDEPENDENTLY PREDICTS CAROTID INTIMA MEDIA THICKNESS IN YOUNG PATIENTS WITH TYPE 1 DIABETES MELLITUS

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Starting from a young age, patients with Type 1 diabetes (T1D) have a high risk of developing cardiovascular diseases (CVD) early in their lives. Carotid intima media thickness (cIMT), a marker for atherosclerosis, was often found elevated in young T1D-patients. The correlation of cIMT and glycemic control, reflected by HbA1c, in young T1D-patients has been inconsistent. We evaluated the relation of cIMT with other CVD risk factors, such as abdominal obesity. Methods: We measured mean and maximum IMT (IMTm) in 77 young adults and adolescents with T1D (age: 19±3 years, diabetes duration (DD) 11±3 years, HbA1c 7.8±1.4 %) and in 25 controls (age: 23±2 years) via ultrasound. We examined CVD risk factors i.e. serum lipids, body-mass-index (BMI), waist circumference (WC), waist-to-height-ratio (WHTR), blood pressure, interleukin-6 (IL6) and high-sensitive-c-reactive protein (hsCRP). Results: T1D-patients had higher systolic blood pressure (SBP) than controls (t=2.669, p=0.009) and larger WC (t: -2.485, p=0.015). There was no significant difference in BMI, serum lipids, hsCRP or IL-6. Age-adjusted IMT did not differ significantly between groups. In diabetics there was no correlation of IMT with HbA1c, SBP, BMI, IL-6 or hsCRP, but IMTm was related to WC (r=0.281, p=0.017) and WHTR (r=0.258, p=0.033). Adjusting for age, sex, Hba1c, DD, SBP, smoking and lipids, WHTR remained the only independent predictor of IMTm (beta=0.329, p=0.024). T1D-patients with increased WHTR (>0.5) (19%) had higher hsCRP-levels (t=-3.316, p=0.013) but did not differ significantly in HbA1c-levels. Conclusion: We found no significant difference in cIMT between controls and young T1D-patients. Every 5th young T1D-patient had an increased WHTR. The association of WHTR with higher IMTm independent of other CVD risk factors suggests the importance of measuring WHTR in young T1D-patients. Independent of glycemic control, young diabetics with abdominal obesity could be the group most at risk for CVD.
Non-alcoholic fatty liver disease (NAFLD) is one of the most common liver pathologies associated with the obesity pandemic and its related metabolic diseases. The abnormal retention of hepatic lipids in NAFLD can be explained, among others, through reduced lipid export from the liver via very-low density lipoprotein (VLDL) particles. To dispose off lipids one of the main pathways the liver takes advantage of is via the secretion of VLDLs. Thus, VLDL secretion rate should never be too low to prevent steatosis. Leptin, a hormone circulating in concentrations proportional to body fat mass, is implicated in regulating hepatic lipid flux. Disrupted brain leptin signaling causes hepatic steatosis independent of changes in body weight and leptin infusion in ob/ob mice increases VLDL secretion. Even though, leptin receptors are also known to exist in the liver, it is thought to mainly act via its brain receptors to regulate energy balance and neuroendocrine functions. Furthermore, recent data from our lab suggest that intracerebroventricular (icv) insulin, which is given directly into the 3rd ventricle of the brain, is capable of increasing hepatic VLDL secretion independent of circulating insulin levels in rodent models, indicating that the brain may play an important role in regulating hepatic VLDL secretion by redistributing lipids into WAT and thus protect other organs, such as the liver or muscle, from ectopic lipid accumulation and lipotoxicity. Brain leptin infusions via stereotaxic cannulae and osmotic mini pumps in Sprague Dawley (SD) rats are performed to study the role of brain leptin in regulating hepatic VLDL secretion. To this point, initial pilot experiments have already been performed. Preliminary data show increased VLDL secretion from the liver independent from changes in food intake. These studies identify brain leptin signaling as an important factor in hepatic VLDL secretion and the findings will advance novel therapeutic concepts in treating NAFLD.

Obesity results from an imbalance between energy intake and expenditure. Humans possess two different adipose tissues: the white adipose tissue (WAT), involved in energy storage and the brown adipose tissue, responsible for energy expenditure by thermogenesis. This process is called uncoupling respiration and is mediated by the tissue-specific uncoupling protein 1 (UCP-1). After the recognition that BAT has a high capacity to dissipate energy, the main focus was on manipulating different metabolic pathways in order to favor energy expenditure as a novel approach to combat obesity. Retinoids have been established as transcriptional mediators of UCP-1 expression and BAT activation. Pharmacologic
stimulation with retinoic acid potently induces UCP-1 expression in brown adipocytes and in BAT depots in mice. Recent preclinical studies suggest vitamin A as a nutrient critically involved in the function of the brown adipose tissue and energy metabolism in rodents. In order to determine the function of vitamin A in the activation of human BAT, we will determine the association between systemic retinol/retinol binding protein (RBP) concentrations and brown fat activity. Therefore retinol and RBP will be measured at baseline (room temperature) followed by [18F]FDG-PET/CT and [18F]FDG-PET/MR scans on study day 1. These tests will be repeated on study day 2 but this time subjects will undergo moderate cold exposure using a cooling vest prior to the analyses. As secondary outcome parameters we will determine cold-mediated changes in retinoid concentrations and potential differences between lean and obese subjects. We will also investigate any association between the basal metabolic rate before and after cold exposure and altered retinoid concentrations. Our results endeavor to define the role of vitamin A in human brown fat physiology and energy balance.

36 GENETICS OF THROMBOTIC MICROANGIOPATHIES


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Introduction: Thrombotic microangiopathies (TMA) classified as hemolytic uremic syndrome (HUS) are characterized by acute kidney injury (AKI), mechanical hemolysis and thrombocytopenia, caused by an excessively activated alternative pathway of the complement system. This is a result of either genetic alterations within complement regulatory proteins or secondary causes. The aim of this study was to systematically investigate our cohort of patients with TMA regarding genotype, triggers of disease episodes, and clinical course of the disease. Methods: Data were analyzed by means of patient records: Demographic and laboratory data, results of kidney biopsies, results of genetic sequencing of complement regulatory proteins and C3, and medical history for triggering factors were analyzed. Results: Out of 76 patients with TMA we classified 23 as atypical HUS (aHUS), one as typical hemolytic uremic syndrome, and 52 as secondary HUS. At onset of disease patients with aHUS had a mean age of 26 years (range: 3-47), 17 were female (74%), and 12 (52%) were kidney transplant recipients. First presentation of 17 patients was AKI, 3 showed hemolysis, two had pre-eclampsia and one HELLP-syndrome. Distinct triggering factors in 14 patients were: infection (n=6), pregnancy (n=5), surgery, diarrhea and high blood pressure (each n=1). Twelve showed a CFH-H3 risk-haplotype and three a MCPggaac risk haplotype; potentially disease-causing mutations were identified in 18 patients (4 CFH, 4 CD46, 4 CFI, 4 C3, 1 CFB, 1 thrombomodulin; no data exists for one patient). Fourteen patients presented with low C3c levels (data unavailable in 7 patients). All patients presented with proteinuria (no data available in five cases). Conclusion: In our cohort of TMA patients we identified 23 cases of aHUS. In 18 patients we identified a mutation within genes of the complement regulatory proteins or C3, whereas 5 subjects showed wild-type sequences despite presenting with a classic phenotype of aHUS.
Following exposure to peritoneal dialysis fluids (PDF), mesothelial cells exhibit features of injury, leading to loss of peritoneal membrane integrity. This damage, as a consequence of non-physiological components of PDF, might be related to inadequate induction of protective cellular stress responses (CSR). Recently our group has shown that a potentially negative regulator of cell survival, glycogen synthase kinase-3 β (GSK-3 β) is upregulated by PDF, resulting in suppression of CSR. Lithium, a well described GSK-3 β inhibitor, augmented the restoration of adequate CSR following PDF exposure in immortalized mesothelial cells. In this study confluent primary mesothelial cells were incubated with commercially available PDF (Extraneal®, Baxter). The effects of PDF with or without added lithium on cell injury were investigated using a lactate dehydrogenase assay. From correspondent samples either total RNA or total protein was extracted. The transcriptome was investigated using gene expression microarrays (Affymetrix) and the biological processes and pathways showing the PDF/Lithium dependence were characterized using the PANTHER database. Significant genes identified in the transcriptomics approach were subsequently verified on the protein level. A dose dependent decrease of toxicity for PDF supplemented with lithium was associated with significantly differential expression of genes responsible for immune system processes and stress responses. The significantly enriched processes contain mainly chaperones, interleukins and cytokines as overrepresented protein classes. Furthermore, the inhibition of GSK-3 β was confirmed for lithium supplemented PDF by differential expression of genes associated with the Wnt-pathway. The observed improvement of cellular stress responses confirms the cytoprotective potential of lithium as intervention in primary mesothelial cells and the pathways identified by transcriptome analysis will be the basis for in-vivo studies.

Peritoneal dialysis (PD) is a renal replacement therapy where the peritoneum is used as semipermeable membrane to remove solutes and water from uremic patients by PD fluids (PDFs). Bio-incompatibility of glucose-based PDFs is responsible for diabetes-like vascular damage occurring in the peritoneal membrane over time on PD, leading ultimately to technique failure. Here we study the effect of PDF supplementation with cytoprotective alanyl-glutamine (AG) on human umbilical vein endothelial cells (HUVEC) following exposure to commercial peritoneal dialysis fluid.
(HUVEC) using proteomics. In an established in-vitro PD model, we compared viability (by LDH release) and proteome of HUVEC exposed to PDF +/- 8 mM AG or control. Using cyanine fluorescent dyes, and two-dimensional difference gel electrophoresis (2D-DIGE), significantly altered spots were identified by mass spectrometry (MALDI-MS). Reduced viability in cells exposed to PDF was attenuated by AG supplementation during PDF exposure thus exerting cytoprotective effect. Out of a common spot pattern of 993 spots, significant abundance changes (p<0.05) were found in 261 and 131 spots in PDF and PDF+AG respectively compared to control. Of those, 104 spots were common whereas 27 spots showed significant changes exclusively following exposure to PDF+AG. Deeper insights on the effect of AG was achieved by direct comparison between PDF and PDF+AG revealing a set of 55 differentially abundant spots (p<0.05). Interestingly, 58.2% of those spots showed restored abundance close to control levels when AG was supplemented. Protein identification suggests a role for AG in attenuating or even reversing cytoskeletal injury caused by PDF exposure of HUVEC (CALD1; VCL; VIM; EZR; CFL1; TCTP; ANXA2), and improving cellular responses to PDF stress (HSPB1; PPIB; TCTP; PDIA6; PDIA3; ERP29; HSP90B1; TXND5; PRDX2; NACA). In summary, this study elucidates potential mechanisms by which AG exerts cytoprotective effects in endothelial cells, offering therapeutic targets to reduce side effects of PD.
39 CARBOXYMETHYL CHITOSAN PREVENTS FORMATION OF BROAD-SPECTRUM BIOFILM

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In this study, we determined the effect of carboxymethyl chitosan (CM-chitosan) on biofilm formation and proposed a mechanism. Gram-positive and Gram-negative bacterial biofilm formation on microtiter plates was prevented 74.6% and 81.6% by CM-chitosan, respectively. Biofilm formation was also severely prevented in dynamic conditions. CM-chitosan inhibits the adhesion of bacteria with an efficiency of >90%. It prevents Gram-positive bacterial biofilm formation at efficiencies of 63.1% and Gram-negative bacterial biofilm formation at efficiencies of 70.6% when CM-chitosan is added at 1 h after biofilm initiation. The prevention of initial bacterial adherence and cell–cell interaction was ascribed to flocculation. The flocculation of Gram-positive and Gram-negative bacteria was 16.7% and 24.6% in the presence of CM-chitosan, respectively. CM-chitosan may neutralize bacterial surface charge and bridge the bacterial aggregates. CM-chitosan decreased 12.9% and 12.8% of the cell surface charge of S. aureus and P. aeruginosa, respectively. CM-chitosan may serve as an antibiofilm agent.

40 THE EFFECTS OF VARIOUS TARGETED DRUGS ON IGE RECEPTOR MEDIATED SIGNAL TRANSDUCTION AND ACTIVATION OF MAST CELLS AND BASOPHILS

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Mast cells [MC] and basophils [BA] are important effector cells in allergic inflammation and express numerous functionally relevant cell surface receptors, including the high affinity IgE receptor [IgERI]. Furthermore, MC express receptor for stem cell factor [KIT] and BA express receptor for interleukin 3 [IL3R]. Tyrosine kinase inhibitors [TKI] are small molecule drugs and most of them have been developed in haematology and oncology. Recently published data show, that PKC412 inhibits IgE-dependent histamine release of cord blood [CB] cell-derived MC and of human BA. Dasatinib also inhibits IgE-dependent histamine release and upregulation of activation-linked surface antigens CD63 and CD203c in human BA. The aim of this study was to examine the effects of various targeted drugs on IgE-mediated histamine release, expression of surface IgERI and/or IgE-dependent upregulation of CD63 and CD203c. We examined human blood BA from healthy donors, CB derived MC and a major MC line model, ROSA-KIT WT, by flow cytometry and histamine release assay. We found that Ibrutinib, a Btk inhibitor and
P505-15, a Syk inhibitor downregulate anti-IgE induced upregulation of CD63 and CD203c and IgE-dependent histamine release on human BA. Additionally, we found that JQ1, a BRD4 inhibitor, inhibits IgE-dependent histamine release and anti-IgE induced upregulation of CD63 and CD203c on ROSA-KIT WT cells. However, when tested on human BA, JQ1 showed no effect on expression of IgERI and anti-IgE induced upregulation of CD63 and CD203c. All in all, our data show that Ibrutinib and P505-15 downregulate anti-IgE induced upregulation of CD63 and CD203c and block IgE-dependent histamine release on human BA. JQ1 downregulates expression of IgERI on ROSA-KIT WT cells but showed no effects on human BA. These results may suggest different downstream signalling molecules in the IgERI mediated activation in BA and MC.

41 L1-RG1 VIRUS-LIKE PARTICLE (VLP) VACCINES DIRECTED AGAINST CUTANEOUS HUMAN PAPILLOMAVIRUSES (HPV)

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Cutaneous HPV of genus Beta are thought to act as adjunct to the main carcinogen UV-light in the development of non-melanoma skin cancer (NMSC) in immunosuppressed patients like organ transplant recipients. Other common cutaneous types, most often HPV1, 2, 3, 4, 27, 57 cause common and palmo-plantar warts, a frequent nuisance in children and burden for health care systems. Licensed HPV vaccines based on major capsid protein L1 virus-like particles (VLP) provide type-specific protection to a limited number of genital HPV types. To develop a vaccine targeting common cutaneous and beta HPV, VLP were repetitively decorated with a cross-neutralization epitope of Beta HPV17’s or HPV4’s minor capsid protein L2 (homologue of HPV16 L2 ‘RG1’) by insertion into the DE surface loop of either HPV16, HPV5, or HPV1, resulting in 16L1-17RG1, 5L1DE-17RG1, or 1L1DE-4RG1 chimeric proteins. Following recombinant baculovirus expression purified VLP + alum-MPL adjuvant were used to immunize NZW rabbits and sera analyzed by ELISA and pseudovirion (PsV) neutralization assays. To fully evaluate cross-neutralization efficacy of L1-RG1 VLP vaccines novel Beta type PsV were generated. Furthermore, in vivo (cross-) protection was analyzed in a murine vaginal challenge model. By ELISA high-titer antisera to RG1 were detected indicating immunogenic RG1 epitope presentation by VLP. PsV assays revealed cross-neutralization against HPV5/8/16/20/23/24/36/92/96 by 16L1-17RG1 VLP, HPV5/20/24/36/92/96 by 5L1DE-17RG1 VLP, and against HPV4 only by the common cutaneous vaccine. Passive transfer of antiserum to 16L1-17RG1 VLP protected mice from challenge with HPV5/16/20 PsV, whereas antisera to 5L1DE-17RG1 VLP conferred cross-protection against HPV20 only, and antisera to 1L1DE-4RG1 VLP against HPV4. Chimeric L1-RG1 VLP vaccination targeting a broad spectrum of cutaneous HPV types is a promising strategy against common warts in children and development of NMSC in organ transplantation.

42 THE ROLE OF HDAC2 IN T CELLS

The interplay of histone acetylation and deacetylation serves as a key regulatory mechanism in T cell development and function by modulating cellular gene expression. The dynamic changes in the acetylation of core histones are mediated through the activity of two large families of antagonistic proteins, namely histone acetyltransferases (HATs) and histone deacetylases (HDACs), which modify chromatin structure through transfer of acetyl-groups to and from lysine residues of histones, respectively. Moreover, HATs and HDACs also act on non-histone targets regulating protein activity, stability, localization and protein-protein interaction. The application of HDAC inhibitors (HDACi) revealed important immunological processes and T cell functions that are dependent on the activity of HDACs. To date, 18 individual HDACs have been identified that act in numerous cellular pathways, frequently through their repressive influence on gene transcription. However, the specific roles of individual HDAC family members in T cells are still subject of ongoing research. Our group previously demonstrated that HDAC1 controls the magnitude of a Th2-type inflammatory response by modulating cytokine expression in effector T cells. During the last years we showed that conditional deletion of HDAC1 in T cells leads to enhanced airway inflammation and increased Th2 cytokine production and that HDAC1 controls antiviral immune responses. However, the role of the highly HDAC1-related HDAC2 protein in T cells is only poorly understood. Here we aim to elucidate the role of HDAC2 in T cells. Results of our ongoing studies will be presented.

43 THE ROLE OF MICRORNA-146A IN INFLAMMATORY ARTHRITIS

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MicroRNA (MiR-) 146a is a key regulator of the innate immune response. Although in late stages of arthritis elevated expression of miR-146a in synovial tissue of rheumatoid arthritis patients was detected, the level of this miRNA is down regulated in early disease, but its role in inflammatory arthritis is yet unknown. We induced K/BxN serum transfer arthritis in wild type and miR-146a-/- mice. As a second inflammatory arthritis model we crossed miR-146a deficient into hTNFtg mice. Disease severity was assessed clinically and histologically. Blood of arthritis animals was analysed by flow cytometry. Serum cytokine levels were measured by Elisa. Absence of miR-146a leads to increased clinical signs of the induced serum transfer arthritis. In line, higher serum levels of the proinflammatory cytokines IL12 and TNF were measured in miR146a deficient compared to wt mice. When we crossed miR-146a-/- mice into hTNFtg mice, while detecting no clinical difference between hTNFtg and miR-146a-/-hTNFtg mice, we found a significant increase in CD11b+ as well as CD11c+ cells in blood of miR-146a-/-hTNFtg mice compared to hTNFtg mice. Histological examination revealed an elevated synovial inflammation in miR-
146a-/-hTNFtg mice compared to hTNFtg mice. Even more striking, miR-146a/-hTNFtg mice displayed a more than twofold increase in local bone destruction which was due to increased generation of osteoclasts in the tarsal joints of the mice. Measuring cytokine levels in serum, we show that IL-1β levels are increased in mice lacking miR-146a. Moreover mRNA expression levels of IL6 and IL-1β in arthritic paws of these mice were significantly elevated in miR-146a-/-hTNFtg mice compared to hTNFtg mice. These results identify an important anti-inflammatory role of miR-146a, which might possibly be exploited for therapeutic purposes.

44 DIVERSITY OF DONOR-SPECIFIC ANTIBODIES INCLUDES MHC-I-SPECIFIC IGE

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The pleiotropic functions of donor-specific antibodies (DSA) are still debated controversially. The presence of DSA is an adverse marker in most, but not all settings. Additionally the function and diversity of DSA-isotypes is insufficiently understood. In particular it is unknown if IgE is induced, which is capable of mediating unique effector mechanisms. Recently we developed a non-MHC antigen-mismatched transgenic mouse model (expressing the antigen Phl p 5 ubiquitously on the cell-surface), in which we found high levels of mismatch-specific IgE after rejection of heart and skin grafts. Here, we studied if donor-specific IgE is induced in an MHC-mismatched mouse model. Tail skin or hearts of Balb/c (H-2d) or C3H (H-2k) mice was grafted in an allo-setting onto naïve B6 (H-2b) or C3H or Balb/c mice (n=6 skin, n=4 hearts). Serum samples were taken pre and post transplantation (TX) at several time-points to analyze H-2Dd-, H-2Kd-, H-2Kk-, H-2I-Ed and H-2I-Ek specific (i.e. murine MHC-I and MHC-II antigens) IgE levels were measured via ELISA by using recombinant MHC monomers provided by the NIH tetramer facility. Serum samples were also used for an in vitro basophil degranulation assay (RBL-assay) to assess if IgE is functional. Via utilization of this novel ELISA we revealed an induction of MHC-I-specific IgE (αI-Ed and αI-Ek) remained undetectable. Additionally we were able to detect basophil degranulation upon cross-linking with recombinant MHC in the in vitro RBL-assay. To the best of our knowledge, this is the first report of MHC I-specific IgE developing upon graft rejection. IgE is functional at the effector cell level in vitro, but whether this isotype plays a pathophysiological role in vivo remains to be assessed.

45 BASOPHIL ACTIVATION TESTS REVEAL THAT THE C-TERMINUS OF BET V 1 CONTAINS IMPORTANT IGE BINDING EPITOPES

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Background: Characterization of IgE epitopes of the major birch pollen allergen, Bet v 1, is essential for the diagnosis and treatment of birch pollen allergy. We showed that the Bet v 1-specific IgE response is polyclonal and highly patient-specific. Moreover, important IgE epitopes are distributed across the whole surface of Bet v 1. However, little is known about the biological activity of IgE specific for different parts of the Bet v 1 surface. Methods: Four Bet v 1-specific surface areas were grafted onto the Bet v 1-related allergen from celeriac, Api g 1. The resulting chimeras, called Api-Bet-1 to Api-Bet-4, were expressed in Escherichia coli and purified by standard chromatographic methods. The aggregation behavior of Bet v 1, Api g 1 and the chimeras was analyzed by dynamic light scattering. Basophil activation tests using whole blood of 6 birch pollen allergic donors were performed with different concentrations (10 pg/mL-1 µg/mL) of Bet v 1, Api g 1 and the chimeric proteins. The percentage of CD63-positive cells in the CCR3+CD123+ population was measured by flow cytometry. Results: Dynamic light scattering confirmed that the recombinant proteins were monomers. Maximum basophil activation ranged from 65% and 100%. Bet v 1 showed the highest and Api g 1 the lowest potency to activate basophils with median concentrations required for half maximum activation of 0,13 ng/mL and 14 ng/mL. Interestingly, Api-Bet-3 revealed the highest basophil activation potency among the chimeras with a half maximum concentration of 0,4 ng/mL compared with 3 ng/mL for the other chimeras. Conclusion: This study shows that grafting of Bet v 1-specific areas onto Api g 1, increased the potency to activate basophils. The fact that Api-Bet-3, which contains the C-terminus and surrounding residues of Bet v 1, exhibited by far the highest potency to activate basophils among the chimeras indicates that important IgE epitopes are located at the C-terminus of Bet v 1.

46 ESTABLISHMENT OF A HUMAN SKIN EXPLANT MODEL TO TEST THE EFFECTS OF TOPICALLY APPLIED SUBSTANCES ON PATTERN RECOGNITION RECEPTOR EXPRESSION IN EPIDERMAL LANGERHANS CELLS

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Allergens initiate allergic responses in susceptible hosts by their unique innate immune activating capabilities. As the identification of allergen-specific pattern recognition receptors (PRRs) in the skin is in its infancy, studies are needed to further define the PRR pathways [Toll-like receptors (TLRs), RIG-I like receptors (RLRs), C-type lectin receptors (CLRs), NOD-like receptors (NLRs)] mediating TH2-biased immune responses to a broad range of allergens via dendritic cells. Keratinocytes and epidermal Langerhans cells (LCs) ascertain a potent skin barrier against environmental threats. As immature LCs express a very limited PRR repertoire in situ we tested whether PRRs can be regulated and activated in LCs upon recognition of specific ligands. For this purpose, we established a human ex vivo skin culture model, displaying a disrupted epidermal barrier, generated by tape stripping. The TLR3 agonist polyribosinic-polyriboctidylic acid [Poly (I:C)] is a well-known potent inducer of a strong inflammatory response in several cell types and was used to establish our skin culture models. Its application onto a
disrupted skin barrier failed to induce TLR3 and protein kinase R (PKR), while it strongly up-regulated melanoma differentiation antigen 5 (MDA5) expression in epidermal LCs. This model will now allow us to test whether and if so which PRRs and signalling pathways will be activated in LCs as well as in keratinocytes after contact with allergens. Our study could help to develop preventive and/or therapeutic strategies to effectively block allergen recognition and the ensuing inflammatory cascade.

47 THE ROLE OF THE TRANSCRIPTION FACTOR MAZR DURING INKT CELL DEVELOPMENT

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Invariant natural killer T (iNKT) cells are innate T lymphocytes that express a semi-invariant T cell receptor. iNKT cells recognize α-Galactosylceramide (αGalCer) presented by the MHC class I related CD1d molecules and are involved in a variety of immune responses (e.g. tumor response, autoimmune disease prevention). We have recently identified the transcription factor MAZR, a BTB/POZ domain containing zinc finger protein, as part of the transcription factor network that regulates CD4+ and CD8+ cell fate choice. However, the role of MAZR in other T cell lineages as well as T cell function remains largely unknown. Here we elucidate the potential role of MAZR during iNKT cell development. Our preliminary data indicate that mice with a T cell-specific selection of MAZR have a reduced number of splenic iNKT cells. Moreover, loss of MAZR results in an alteration in iNKT lineage decision towards distinct subsets (e.g. NKT1, NKT2 and NKT17). By using biochemical as well as genetic approaches we are currently addressing how MAZR is integrated in the transcriptional regulation of iNKT development. Furthermore, in vivo iNKT cell responses in the absence of MAZR will be tested.

48 A genome-scale collection of gene deletion mutants in C. glabrata

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Within the last decade, there has been a steady increase of invasive fungal infections in immunocompromised patients. The most common of these infections are caused by Candida spp., which has a high mortality in its disseminated form. C. glabrata (C.g.) now is the second most frequent cause and accounts for 15 - 20 % of all cases of Candidiasis. Importantly, C.g. is inherently tolerant to azole antifungals when compared to most other Candida spp. This is of clinical importance because of the wide use of azole therapy in fungal infections. Moreover, C.g. is unable to form true hyphae or secrete proteases, which are considered important virulence factors of C.a. These differences, as well as the high tolerance to conventional fungal therapies, leave the nature of virulence factors largely unknown. To identify candidate virulence and drug resistance factors, we initiated the construction of a large-scale collection of C.g. deletion mutants. This collection enables studies on the molecular functions of genes
implicated in virulence and drug resistance, as well as those modulating signaling pathways and stress response. Hence, we use a reverse genetic approach to generate a bar-coded C.g. gene deletion collection, which is subsequently analyzed in vitro for their sensitivity to different environmental stress conditions including heat, pH and osmotic stress, as well as a variety of other conditions including antifungal drug susceptibility. The collection now comprises more than 700 deletion strains. We shall present a comprehensive data set on the phenotypic profiling of the deletion collection in comparison to data from clinical patients isolates. Interestingly, a number of C.g. clinical isolates display high-level resistance to azoles (Fluconazole, Voriconazole and Posaonazole) as well as echinocandins (Caspofungin).

49  RESVERATROL AND THE SYNTHETIC RESVERATROL DERIVATIVE FEHH: A COMPARATIVE STUDY IN T- CELLS

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Resveratrol is a natural occuring polyphenol produced in plants (red grapes, raspberries, blueberries). The potential positive health effects of resveratrol are for example its anti-inflammatory, anti-carcinogenic and anti-oxidant activity. The resveratrol derivative FEHH was chemically synthesized and promises to be more stable and lipophilic than the original. This possibly might prevent oxidation by atmospheric oxygen and enables an increased uptake into the cell. The aim of this work was to compare the mode of action of these substances in Jurkat T- cells. Jurkat T- cells were activated with PHA and PMA in the absence or presence of increasing concentrations of resveratrol or FEHH. IL-2 promoter driven luciferase activity was measured after 6h. For the detection of cytokine expression (IL-2), the cells were stimulated for 24 h with PHA/PMA-/+ resveratrol or FEHH. For monitoring of apoptotic cell death Annexin-V/7-AAD staining was performed. In order to evaluate whether the results in Jurkat T- cells can be transferred to peripheral T- cells we perform first experiments to determine the effects of both compounds on cytokine secretion and proliferation. Therefore, CD4+ T- cells were pre- incubated with different concentrations of resveratrol or FEHH and were activated using agonistic anti-CD3/anti-CD28 antibodies. IL-2, IFNγ and TNFα was measured in cell culture supernatants. The dilution of fluorescent cell proliferation dyes were detected by flow cytometry. We showed that both resveratrol and FEHH decreased luciferase activity and IL-2 secretion in Jurkat T- cells. However, FEHH was more effective than resveratrol considering the inhibitory effects on IL-2 expression. In comparison to resveratrol, FEHH already induced apoptotic cell death at lower concentration in Jurkat T-cells. We demonstrated that resveratrol and FEHH inhibit the secretion of the cytokines IL-2; IFN-α/β and TNF-α as well as the proliferation of CD4+ T- cells.

50  SCHLAFEN GENE FAMILY MEMBER 12 (SLFN12) IS REQUIRED FOR HUMAN RHINOVIRUS (HRV) INFECTION OF HEla-OHIO CELLS

The mammalian Schlafen gene family (SLFN) has been implicated in the regulation of cell proliferation and differentiation as well as control of viral replication, such as of human immunodeficiency virus (HIV), vesicular stomatitis virus (VSV) and murine gammaherpesvirus 68 (MHV-68). SLFNs are inducible by type I interferons (IFNs). Human rhinovirus (HRV) infections are one of the most frequent upper respiratory illnesses in humans worldwide and result in mild diseases like the common cold. HRV is human pathogenic and its replication takes place only in the cytosol of epithelial cells. Six human SLFN genes (SLFN5/11/12/12L/13/14) have been identified, whereby SLFN12 is the only human SLFN predicted to be located in the cytosol. Previously we found that SLFN12 is highly up-regulated in dendritic cells (DCs) upon HRV stimulation. Therefore, we hypothesized that SLFN12 might be involved in the regulation of HRV infection in human epithelial cells. Retroviral overexpression of SLFN12 in HeLa-Ohio cells led to cell death and cells could not be further used for HRV infection experiments. Gene silencing impeded cell proliferation of HeLa-Ohio cells, but did not affect cell viability. Moreover, silencing did not alter IL-6 production or expression of related cell markers such as intercellular adhesion molecule 1 (ICAM-1), myxovirus resistance gene A (MxA) and transferrin receptor (CD71). Most importantly, replication of both HRV14, which uses ICAM-1 for cell entry, and HRV2, which enters via low density lipoprotein receptors (LDLR), was impaired in HeLa-Ohio cells upon SLFN12 silencing at the mRNA as well as at the protein level. Thus, SLFN12 is required for efficient HRV replication in HeLa-Ohio cells. There is indication that SLFN12 is a novel cellular host factor for HRV replication, which might be involved in HRV translation as well as in transcription. Moreover, SLFN12 seems to be a rheostat that regulates the proliferation and viability of epithelial cells.

51 THE INFECTIOUS DOSE OF CHLAMYDIA CAVIAE MODULATES THE IMMUNE RESPONSE PROFILES IN REPEATED CONJUNCTIVAL INFECTION IN GUINEA PIGS


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Inclusion conjunctivitis in guinea pigs (GPs), caused by Chlamydia caviae (ChC) is a well-known animal model for studying ocular disease trachoma. Our goal was to investigate protective immunity that develops after repeated conjunctival infections with different infectious doses of ChC. GPs were infected conjunctivally by applying 104 or 106 Inclusion Forming Units (IFUs) of ChC. Infection was repeated two more times in intervals of 6 and 12 weeks, respectively. Animals were monitored daily for the signs of ocular pathology. In each infection (IN), at days 0 (prior-infection), 4, 7, 14 and 21 post-infection,
conjunctival swabs and blood were collected to quantify chlamydial IFUs and ChC-specific sera IgG titers, respectively. 2nd IN with the lower dose completely decreased infection course and ocular pathology. The adaptive response was altered, with increased ChC-specific sera IgG titers at all-time points and increased capability of these antibodies to bind to and neutralize the infection of ChC in vitro. However, only partial protection was seen in GPs infected with higher dose after 3rd IN. The observed capacity of animals infected with the lower dose to better control the infection by limiting an inflammatory environment and the resulting clinical pathology is important in modulating the adaptive immune response.

52 PARASITE DERIVED IMMUNOMODULATORS FOR PREVENTION AND THERAPY OF ALLERGY


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The inverse relationship between infection with certain parasites and a reduced incidence of allergic disease has been repeatedly confirmed in numerous studies. The so called “hygiene hypothesis” has opened a new field in allergy research aiming at identifying parasite-derived immunomodulators. We have previously shown that infection with certain protozoa and helminths, such as Toxoplasma gondii or Oesophagostomum dentatum prevented allergic immune responses and airway inflammation in a mouse model of type I allergy. In continuation of these studies we now demonstrate that the application of extracts from these parasites also reduce airway inflammation along with decreased levels of IL-5 and eosinophils in bronchoalveolar lavage. Moreover, we show that upon heat-inactivation the suppressive effect of O. dentatum is stable, whereas T. gondii extract loses its immunomodulatory potential. Further aim in this study therefore is to identify, characterize and produce T. gondii- and O. dentatum-derived molecules with these immunomodulatory properties. For this purpose the extracts of both parasites are being fractionized and biochemically characterized with different techniques such as normal- and reversed-phase HPLC, 2D gel electrophoresis, followed by MALDI-TOF-MS and ESI-MS/MS in order to identify specific compounds with immunomodulatory/anti-allergic properties. The most promising candidates will be purified/produced and tested in vitro and in vivo aiming to use them as adjuvants in future allergy vaccines.

53 COMPARISON OF SUBCUTANEOUS AND INTRA MUSCULAR APPLICATION OF A TICK-BORNE-ENCEPHALITIS VACCINE REVEALS COMPARABLE TBE SPECIFIC HUMORAL IMMUNE RESPONSES BUT A HIGHER LOCAL REACTOGENICITY PROFILE


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Studies to determine the optimal vaccination route are being performed during the licensing studies for vaccines evaluating immunogenicity and reactogenicity. For the TBE vaccine only the intramuscular (i.m.) route is licensed for routine vaccination. However in various situations, either because of medical indications, accidental application or due to a lack of sufficient muscular tissue, these vaccines might be rather applied via the subcutaneous (s.c.) route. As no information is available, if TBE vaccines can be subcutaneously applied with the same immune responsiveness as the i.m. application, the following study was performed: 116 female and male participants were included and randomly selected for either i.m. or s.c. injection of FSME-Immun®. Blood was collected at 4 different time points after vaccination for antibody titer analysis. Before and 7 days after vaccination PBMCs were isolated for FACS analysis of lymphocyte subpopulations and cytokine production upon antigen restimulation. Monitoring of potential side effects for 7 days post vaccination revealed a significantly higher occurrence, duration and intensity of local reactions after s.c. vaccine application compared to i.m. vaccination. In contrast the occurrence of most systemic side effects was either the same or lower after s.c. vaccination. Cytokine production after antigen specific restimulation showed a higher increase of IFN gamma and IL10 after s.c. vaccination, however the induction of neutralizing TBE specific antibody production was the same after both vaccination routes. FACS analysis of B and T cell subpopulations showed a relative increase of CD4+ T-cells after i.m. and not after s.c. vaccination and a comparable relative decrease of regulatory T-cells after both vaccination routes. In conclusion, this study indicates that, both vaccination routes lead to comparable protective immune responses. Nevertheless increased side effects after s.c. vaccination needs to be mentioned.

54 PRIMARY PREVENTION OF ALLERGIC AIRWAY INFLAMMATION BY PERINATAL ADMINISTRATION OF A PROBIOTIC ESCHERICHIA COLI STRAIN IN MICE


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Clinical studies have demonstrated that application of the probiotic Escherichia coli strain O83 effectively prevented the development of allergic diseases in children with familial predispositions. The aim of our recent study is to investigate the capacity of perinatal exposure to E. coli O83 to prevent allergic airway inflammation in a mouse model of Type I allergy. In particular, we will test whether perinatal exposure to this probiotic strain induces epigenetic changes associated with allergy-protective effects in the offspring. As prerequisite for this, we characterized the immunomodulatory properties of E. coli O83 in vitro. Mouse splenocytes and bone marrow-derived dendritic (BMDCs) cells were incubated with different concentrations of bacteria. This led to significantly increased levels of IFNγ and IL-12 in supernatants of splenocytes and BMDCs, respectively, suggesting that this strain induces Th1-biased
immune responses in vitro. In order to analyze potential histone modifications in T helper cells, we established the technique of chromatin immunoprecipitation by using an in vitro and in vivo model of CD4+ T cell polarization. In the context of this study we are now characterizing potential cellular, molecular and epigenetic mechanism that might be involved in the prevention of allergy by probiotic bacteria.

55 ELEVATION OF CD19^+CD21^{low} B-CELLS IN PATIENTS WITH CHRONIC GRAFT-VERSUS-HOST-DISEASE: A VALIDATION STUDY IN TWO INDEPENDENT PATIENT COHORTS


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Chronic graft versus host disease (cGVHD) is a serious complication of allogeneic hematopoietic stem cell transplantation (HCT). To date, there is no validated biomarker that would allow objective diagnosis of active cGVHD. Here, we validated B-cell subpopulations including CD19^+CD21^{low} B-cells in the peripheral blood (PB) in two independent patient cohorts after allogeneic HCT as biomarkers for diagnosis of active cGVHD. At the Medical University of Vienna (MUV) 155 patients with a median age of 42 (range, 18-73) years including 117 with active cGVHD and 38 no-cGVHD were analyzed. Fresh PB whole blood cells were analyzed by flow cytometry. Median duration of cGVHD prior to study enrollment was 3 (range, 0-47) months. The second patient cohort consisted of 50 patients with a median age of 50 (range, 15-66) years from the National Institutes of Health (NIH) including 39 with active cGVHD and 11 no-cGVHD. Analyses were performed on frozen samples obtained at study enrollment. The same gating strategy was used for flow cytometry in both cohorts. In both patient cohorts percentages of CD19^+CD21^{low} B-cells were significantly higher in patients with active cGVHD (MUV: 26.41 vs. 9.05, p<0.001; NIH: 12.09 vs. 5.08, p = 0.024). A significant elevation in relative numbers of CD19^+CD21^{low}CD38^{low} B-cells (MUV: 11.64 vs. 3.96, p < 0.001; NIH: 9.11 vs. 2.53, p = 0.030) and CD1^+CD21^{low}CD27^{low} B-cells (MUV: 20.91 vs. 7.94, p < 0.001; NIH: 10.67 vs. 4.27, p = 0.028) was observed in patients with active cGVHD compared to the no-cGVHD, respectively. Absolute and relative numbers of various B-cell subpopulations differed between the two institutions due to the fact that clinical patient characteristics and duration of cGVHD differed and fresh vs frozen samples were analyzed. Nevertheless, our results are promising and show significant association of CD19^+CD21^{low} B-cells and their subsets with active cGVHD, confirming our previous findings.

56 MOLECULAR ANALYSIS OF MAZR FUNCTION IN CD4+ HELPER T CELLS


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Transcriptional and epigenetic mechanisms play a key role in the regulation of T cell development and function. We identified the BTB zinc finger protein MAZR (also known as Patz1) as an important regulator of Cd8 gene expression in DN thymocytes and revealed its essential role in CD4/Cd8 lineage choice of DP thymocytes. However, the role of MAZR in peripheral T cell development has not been elucidated so far. Also, it is not known what role MAZR plays in the tightly regulated network of T helper differentiation. To comprehensively analyze the in vivo and in vitro function of MAZR in CD4+ T cells, we are employing conditional gene targeting approaches (using the Cd4-Cre delete strain) as well as gain-of-function studies using retroviral-mediated overexpression strategies. Preliminary results suggest a role for MAZR in modulating Th17/Treg differentiation and function. Data from our ongoing experiments will be presented. Abbreviations: MAZR (Myc-associated zinc finger related factor); CD (Cluster of Differentiation); BTB (broad complex tramtrack bric-a-brac); Patz1 (POZ-, AT hook-, and zinc finger-containing protein 1); DN thymocytes (double-negative thymocytes).

57 ALANYL-GLUTAMINE IN PERITONEAL DIALYSIS FLUID ENHANCES PERITONEAL IMMUNOCOMPETENCE IN MICE WITH PERITONITIS

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Peritoneal dialysis (PD) is an alternative to haemodialysis and the only modality to treat end-stage renal failure in newborns and infants. PD fluids (PDF) increase inflammation and hamper immune defenses in the peritoneal cavity. Peritonitis represents a relevant factor of morbidity and mortality during PD. Parenteral administration of alanyl-glutamine (AG) has been shown to improve clinical outcome in critically ill and sepsis patients. Aim of the study was to analyze the effect of AG in PDF on peritoneal cells following exposure to combined cytotoxic and infectious stress. C57BL/6 mice were exposed to 2x/day intra peritoneal injections of PDF in combination with 107 colony forming units Staphylococcus epidermidis on day 2 and day 4. PDF was applied with or without 8 mM AG. 4 mice were used as controls. Body weight and pain status were assessed every day. After 9 days, all mice were subjected to a 1h PD dwell. Peritoneal and blood cell counts and cytokine levels were determined. For functional measurements of immunocompetence, fresh effluent was ex-vivo stimulated with lipopolysaccharide (LPS) and analyzed for cytokine release. Local inflammation tended to be more activated in the PD than in the PD+AG group. Basal levels of interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α) in peritoneal effluents were lower with AG. Ex-vivo LPS stimulation of peritoneal cells resulted in increased IL-6 and TNF-α release in controls that were depressed in the PD group and restored in the PD+AG group, indicating improved cellular immunocompetence with AG in PDF. This study supports the concept of increased inflammation and hampered immunocompetence in a model of combined intraperitoneal cytotoxic and infectious stress following exposure to infected PDF reflecting a clinical relevant situation in PD. Addition of AG to PDF attenuated inflammation and restored immunocompetence thus providing first evidence that immunomodulatory effects of AG might be transferred into PD.

58 NEUTROPHIL SUBSETS IN A HUMAN MODEL OF ENDOTOXIN-INDUCED SYSTEMIC INFLAMMATION
Dynamics of neutrophil subsets under conditions of systemic inflammation induced by intravenous application of bacterial endotoxin were studied in 7 male volunteers aged 18-35. The neutrophil subsets were phenotypically defined as follows: quiescent CD66b+ CD62L++ CD16++, activated CD66b+ CD62L+ CD16++, newly released CD66b+ CD62L++ CD16+. In addition, CD66b+ CD54+ neutrophils were considered to have migrated into tissue and returned to circulation. Furthermore, we measured neutrophil-platelet aggregates defined by CD66b+ CD41+. E.coli lipopolysaccharide (LPS) at a dose of 2 ng/kg body weight was injected into the peripheral vein and blood was drawn from the contralateral arm at baseline and 1 to 24 h post injection into hirudin tubes and fixed immediately. Immunofluorescent staining was performed with monoclonal antibodies against target antigens CD66b (carcinoembryonic antigen-related adhesio molecule 8), CD16 (Fcγ receptor III), CD62L (L-selectin), CD54 (ICAM-1), CD41 (αIIbβIII). Samples were measured by flow cytometry. Total neutrophil counts dropped to 43% at 90 min post injection with a subsequent steep rise which peaked at 4 h with 209% of baseline values. The neutrophil subset newly-released from bone marrow showed highest levels at 2 h and comprised 24% of the total neutrophil pool. Activated neutrophils peaked at 4 h constituting 5% of the total pool. The neutrophil distribution returned to the baseline state at 24 h. No significant changes were observed in CD54 expression of neutrophils. Neutrophil-platelet aggregates showed a minor increase at 6-8 h post LPS injection. The established protocol was suited to detect the rapid loss of neutrophils during human endotoxemia (90 min), the subsequent release of neutrophils from bone marrow (peak at 2 h) and the appearance of an activated neutrophil subset (peak at 4 h). This method will be used to study neutrophil subsets in vascular disease patients.

59  MECHANICAL FORCES IN T-CELL ANTIGEN RECOGNITION

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The recognition of antigenic peptide/MHC complexes (pMHC) on antigen presenting cells (APC) by T-cells is mediated by the T-cell receptor (TCR) and leads to the formation of an immunological synapse. This process is remarkably specific, sensitive and efficient but up to this date the underlying mechanisms are only poorly understood. There is mounting evidence that mechanical forces acting on the TCR are indeed instrumental in TCR-ligand discrimination and TCR-mediated signaling. To investigate this in sufficient detail, we will attach force sensors to pMHCs, which will be embedded either on a well-defined functionalized planar lipid bilayer system or on the surface of live APCs. Fluorescent dyes on both ends of the sensor will serve as donor and acceptor for Förster resonance energy transfer (FRET). We expect high FRET values when no tension is applied and the sensor is relaxed. However, cell-imposed forces on the
TCR should stretch the sensor and reduce FRET due to larger distances between the FRET dye pair. To correlate measured FRET to corresponding forces we will calibrate sensors with optical tweezers. We intend to perform FRET measurements in bulk to map forces within the immunological synapse and also to perform single molecule experiments to assess the true molecular force dynamics. To determine the role of TCR-imposed forces in T-cell triggering and ligand discrimination we will correlate them with the stimulatory potency pMHCs and simultaneously imaged downstream signaling. In summary, we expect to visualize TCR-imposed forces most directly, establish them as a crucial factor in T-cell antigen recognition. We expect that many of the lessons learned will be applicable to studying other cell-cell interactions as they might be relevant in the activation of other immune cells, cancer progression, infection biology and neurosciences.

60 STUDY OF ELECTRICAL ACTIVITY AND VESICULAR EXOCYTOSIS DURING ACTIVATION OF HUMAN T LYMPHOCYTES

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Electrical activity of the plasma membrane is essential for cellular functions. For example, changes in membrane potential of neurons and muscle cells play a major role in the spread of neuronal excitation. However, much less is studied the role of the electrical activity of the plasma membrane upon activation of T lymphocytes. The main theme of our project is to contribute to the understanding of the molecular machineries at the plasma membrane of T cells in the first moments after stimulation of the T-cell receptor (TCR). In our work, we combine biophysical techniques (patch clamp), with confocal microscopy, flow cytometry and biochemical approaches. The study includes also an analysis of vesicular exocytosis, the importance of which in the T-cell activation has been more and more appreciated. We are just in the beginning of our project, however, our first results have revealed the role of CD222 (the mannose 6-phosphate/insulin-like growth factor 2 receptor - M6P/IGF2R), the major endosomal transporter and vesicular protein, in controlling the electrical activity of the plasma membrane upon activation of T lymphocytes. This work was supported by the FWF - Austrian Science Fund (P22908) and VEGA - Slovak Grant Agency (2/0063/14).

61 INTERACTION OF INTESTINAL AND AIRWAY EPITHELIA WITH GAD M 1, THE MAJOR ALLERGEN FROM ATLANTIC COD

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Background: Atlantic cod is among the most important of all commercial fishes. Although considered a healthy food, fish can induce IgE-mediated food reactions in sensitized individuals, leading to serious health problems. Sensitization and elicitation of the symptoms occur mainly by allergen exposure via gastro-intestinal tract, but also by inhalation of fish allergen present in wet aerosols. Absorption of fish allergens is very rapid resulting in an onset of allergic symptoms shortly after exposure. Gut and airway epithelia as well as the interaction of allergen and epithelia therefore play a pivotal role in the immune responses. The vast majority of allergic reactions to fish are caused by the major fish allergen parvalbumin. Objectives: We aim to explore interactions of human intestinal as well as bronchial epithelial cells with Gad m 1, a parvalbumin and the major allergen from Atlantic cod in order to understand the high allergenic potential of Gad m 1, i.e. rapid induction of severe symptoms at low concentrations. Interactions of Gad m 1 with epithelium may include transcellular transport, binding and release of inflammatory mediators or disruption of epithelial tight junctions. Furthermore, we will explore influence of additional factors (such as lipids found in allergen source) on these pathways.

Methods: We use Caco-2 and 16HBE14O-cells as in vitro models of human intestinal and bronchial epithelial cells, respectively. Later on, primary cells shall also be used. Results: We currently explore the type of interaction (binding and/or uptake) of Gad m 1 with the cells using fluorescently labelled allergen and fluorescence microscopy. Our initial data show that apical exposure of Caco-2 and 16HBE14O-cells to Gad m 1 results in binding of the allergen to the apical (luminal) plasma membrane of the cells.

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62  RECOMBINANT PROBIOTIC BACTERIA EXPRESSING ALLERGIC-CHIMERS FOR THE NEONATAL PREVENTION OF POLYSENSITIZATION

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It is well recognized that allergic individuals are at risk to develop multiple allergies and such polysensitized individuals are difficult to treat by conventional therapeutic measures. We have recently established mouse models of poly-sensitization and demonstrated that allergic poly-sensitization can be suppressed by mucosal treatment with novel allergen chimers in adult mice. With respect to neonatal interventions, we previously showed that colonization with recombinant probiotic strains expressing the
allergen Bet v 1 successfully prevents allergic responses. In order to investigate the concept of neonatal colonization with recombinant probiotic bacteria for prevention of allergic multi-sensitivities, our first aim is to construct a recombinant probiotic bacteria, constitutively expressing a birch (Bet v 1) and grass pollen (Phl p 1 and Phl p 5) chimer. We have successfully cloned Lactobacillus plantarum with birch and grass pollen chimer. To test if this recombinant LABs can be used to prevent poly-sensitization the strain will be used for the neonatal colonization in (a) conventional mice and in (b) germ-free mice prior to sensitization with allergens. Apart from testing the effects of these treatments, interaction of recombinant LABs with the host immune system will be studied.

63 Evaluation of TLR2 R753Q in Heart Transplant Recipients as Risk Factor for Cytomegalovirus Infection

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Toll-like receptors (TLR) are integral part of innate immunity, which is the first line of defense against infectious pathogens, such as human cytomegalovirus (CMV). The innate immune response to CMV is initiated by ligation of the viral envelope glycoproteins B (gB) and H (gH) with TLR2 and leads to a cascades of intracellular signaling events for elimination of the viral pathogen and also the induction of protective adaptive immunity. The TLR2 single nucleotide polymorphism (SNP) Arg753Gln has a frequency of 6.8% in the general population and was associated in several studies with a predisposition to a variety of infectious diseases. A single study identified in a small cohort of liver transplant patients an association between this SNP and a more severe course of CMV disease. In this study we tested for the distribution of this SNP in samples from healthy blood donors (n=281) in relation to their CMV-serostatus and from heart transplant recipients (HTX) (n=174) in relation to occurrence of CMV infection. In addition, data obtained in HTX patients were stratified for multiple other molecular and clinical characteristics such as genetic mismatches between donor and recipient, other opportunistic infections, human leukocyte antigen (HLA) -mismatch, graft rejection and survival. We didn’t find any change of the SNP distribution between the healthy control group (94.24% wild type, 5.18% heterozygous, 0.58% homozygous) and the HTX patients (93.95% wild type, 5.69% heterozygous, 0.36% homozygous). Also clinical investigations did not show a correlation of this SNP and the course of disease, only one patient developed a severe graft vasculopathy, but has a wild genotype. We only found one patient that was homozygous for this SNP, but the patient developed only a mild viremia. This SNP is too infrequent in the Austrian population to start a risk stratification and identification of effective preventive strategies.
64 Expression and regulation of Schlafen family members in primary human monocytes, monocyte-derived dendritic cells and T cells

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The Schlafen (Slfn, SLFN) family has originally been described as a gene family involved in thymocyte development and cellular growth control in the mouse. However, new evidence indicates important roles for Slfns in immune processes including control of viral replication and immune cell quiescence. Although the Slfn family has been conserved across mammalian evolution, rapid evolution of Slfns suggests functional differences between mouse and man. To date, most research has been focused on the characterization of Slfns within the murine system or in cell lines. Since little is known about SLFNs in primary human immune cells, we set out to analyze the expression and regulation of the six human SLFN genes in monocytes, monocyte-derived dendritic cells (moDCs) and T cells. We activated these cells using various stimuli followed by analysis of SLFN gene expression via quantitative real-time PCR. We demonstrate that human SLFNs are regulated during the differentiation of moDCs and are inducible by stimuli that induce autocrine type I interferon signaling in moDCs. Furthermore, we report moderate downregulation of several SLFN family members during the activation of primary human T cells. Comparison of SLFN gene expression across the three cell types shows high expression levels for SLFN11 in monocytes and moDCs and high SLFN5 expression in T cells indicating functional importance within these cell types. In conclusion, we show for the first time expression profiles of SLFN family members in primary human immune cells.

65 Marginal role of phosphorylations on CMV tegument phosphoproteins

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Human cytomegalovirus (CMV) is an important viral pathogen with worldwide distribution, causing congenital infection with an overall birth prevalence of 0.64% in the developed world and seropositive rates ranging from 60 to over 90 percent dependent on socio-economic status and country. Individuals with a suppressed, impaired or yet undeveloped immune system are at serious risk of CMV associated
disease and symptoms. The phosphorylation of proteins has important regulatory functions and is tightly regulated with counteracting forces of kinases and phosphatases. Important tegument proteins of the human cytomegalovirus have been shown to be phosphorylated and these phosphate modifications are thought to have a major impact on the protein mass. However, enzymatically functional cellular phosphatases are also enclosed in the tegument, and initiate a cell-wide hypophosphorylation state within minutes after viral entry. In addition, CMV induces an upregulation of these cellular phosphatases despite important cell cycle regulators which are the native targets of these phosphatases have been shown to be phosphorylated during viral infection. To examine the role of phosphate modifications on the protein mass and the host immune response, we performed phosphatase treatment with the recombinant and viral tegument proteins pp150 and pp65 and further evaluated the amount and position of phosphorylated residues by mass spectrometry. Immunoblotting showed only a minor impact of phosphate modification on the protein mass and suggested a marginal presence of phosphorylated residues, which we could confirm by use of mass spectrometry. Moreover, we found no significant impact of dephosphorylation on the immune response. In conclusion, our data suggest that the amount of phosphorylation on CMV tegument proteins is largely overestimated and plays only a minor role in the immune response.

66 Impacts of zoonotic chlamydia co-infections on dynamic of chlamydia trachomatis and host cell responses

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Trachoma is a chronic follicular keratoconjunctivitis caused by repeated infection of conjunctiva with the ocular serovars of Chlamydia trachomatis. There are significant differences in the development of symptoms and patterns of disease after infection of the conjunctiva by C.trachomatis. The role of opportunistic bacteria and zoonotic Chlamydiaceae species which are present in the conjunctiva of trachoma patients and the impacts of these bacteria on the dynamic of C. trachomatis infection are poorly understood. Using a suitable Bio-bank from Center of Ocular Vaccine (OCUVAC) at the Medical University of Vienna, which contains samples of trachoma patients and age-matched healthy individuals from different trachoma endemic areas in Africa, we are planning to investigate variations in the zoonotic species of C.trachomatis that may affect the mechanism of chlamydia infection. The influence of the most striking zoonotic Chlamydiaceae species found by sequencing method will then be analyzed in vitro regarding a series of experiments to investigate if the dynamic of C.trachomatis infection could be affected from concurrent infection with these bacteria. Also the differences of cytokines and biomarkers secretion and expression profiles after concurrent infection of two ocular cell lines with C.trachomatis and zoonotic Chlamydiaceae species in comparison to single infection of C. trachomatis will be studied. In the current proposal we intend to investigate a possible association of zoonotic Chlamydiaceae species with dynamic of C.trachomatis infection and host cell responses which could lead to new mechanisms of prophylaxis and also new therapeutic ways to control the infection.
PTEN-deficiency in myeloid cells alters tumor immune surveillance in a murine model of inflammation driven colon cancer

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Macrophages and antigen-presenting cells represent the cellular part of the innate immune system. These cells are the first line of defense against invading microbes, as they produce cytokines, activate other leukocytes and are implicated in the resolution of inflammatory processes and tissue remodeling. But they have been found to contribute also to tumor progression as they undergo phenotypic conversion from an inflammatory state into an alternatively activated, tumor tolerating state. We could show that the PI3K/PTEN signaling pathway plays a role in this fate decision process. Deletion of PTEN in myeloid cells leading to sustained PI3K activation results in an M2-like phenotype characterized by an increase in M2-markers and release of anti-inflammatory factors.

In acute models of inflammation this anti-inflammatory state in myeloid cells is beneficial, but a diminished innate immune response could be detrimental during tumor development. We addressed this question by applying a model of inflammation-driven colon cancer in myeloid-PTEN deficient mice. PTEN⁶/⁶ LysM cre conditional knock-out mice showed an increased tumor incidence and progression and increased mortality during CA development. Isolated myeloid cells exhibited an up-regulation of M2-marker genes and a down-regulation of pro-inflammatory cytokines. Moreover, we found an increase in immune-regulatory innate cells in the secondary lymphoid organs. T-cells isolated from myeloid-PTEN deficient mice had decreased cytokine production as well as a reduced proliferative potential ex vivo. Therefore we suggest that myeloid PTEN deficiency leads to a hypo-responsiveness in T-cells allowing for unimpeded intestinal tumor growth.

Taken together this study highlights the importance of the PI3K/PTEN signaling axis in myeloid cells in tumor immune surveillance and supports the idea that PI3K inhibitors currently used in clinical settings may have additional functions beyond tumor cell targeting.

Determining the Influence of Cell Adhesion and Costimulation on T-Cell Antigen Recognition

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T-cell antigen recognition is central to adaptive immunity. Even though all major molecular players are identified, we understand only vaguely how T-cells manage to detect traces of antigen with high efficiency and without delay. This is at least in part because binding between the T-cell receptor for
antigen (TCR) and its ligand, the peptide-loaded MHC molecules (pMHC), takes place within the immunological synapse, the contact between T-cell and antigen presenting cell (APC). TCR-pMHC binding dynamics are hence heavily influenced by synaptic geometrical constraints, cellular forces, cell adhesion and costimulation, which tune T-cell activation outcomes. To account for these non-linear factors, we are devising a live-cell imaging system with single molecule resolution, which will allow us to visualize and quantitate synaptic protein-protein interaction, nanoscale structures and plasma membrane domains in situ. TCR-transgenic T-cells of various differentiation stages will be confronted with planar glass-supported lipid bilayers, which serve as surrogate APC as they are functionalized with pMHCs and accessory molecules in a defined manner. We will in particular determine the influence of costimulation (as mediated by B7-1, B7-2, ICOSL) and cell adhesion (through CD48 and ICAM-1) on synaptic TCR-pMHC binding with the use of a Förster resonance energy transfer approach. To evaluate TCR-pMHC binding functionally we monitor T-cell signaling simultaneously. We will furthermore apply superresolution microscopy and single dye tracing approaches to (co-) localize each of these molecules and their binding partners on the T-cell below the diffraction limit of visible light (20 nm). Moreover, we will synchronize TCR-binding with the use of caged TCR-ligands as a means to monitor initial events in T-cell recognition. We expect that this approach of direct visualization will prove key for the understanding of T-cell antigen recognition.

69 Toll-like receptor 7 and 9 in the pathogenesis of inflammatory autoimmune arthritis

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Release and insufficient removal of endogenous nucleic acids may trigger autoimmune reactions important in the initiation of rheumatoid arthritis (RA). Nucleic acid-sensing molecules such as the endosomal Toll-like receptors (TLRs) 7 and 9, have been linked to autoimmune processes but their role in RA is less clear.

We aimed to study the role of TLR7 and 9 in the pathogenesis of arthritis by antagonizing or stimulating them in rats with pristane-induced arthritis (PIA).

Arthritis was induced with pristane. Antagonists or agonists for TLR7 and 9, a control sequence or placebo were applied. Treatment was started before disease induction. Arthritis was scored using established scoring systems, inflammation and bone erosion were quantified by histological analysis. Serum cytokine levels were measured by ELISA.

Treatment with the TLR9 antagonist reduced arthritis severity significantly, whereas a slight aggravation was observed in animals treated with the TLR7 antagonist. Inhibition of TLR9 led to reduced bone erosion and it was slightly aggravated in rats treated with the TLR7 inhibitor. IL-6 serum levels were reduced after treatment with the TLR9 antagonist. These effects were only seen when the inhibitor was applied before disease onset. When treatment with the antagonists was started at disease-onset neither disease severity nor bone erosion was affected.
Treatment with a TLR9 agonist had no significant effect on disease severity. Disease was aggravated in animals treated with the TLR7 agonist. This effect was more pronounced than that observed in experiments with the TLR7 antagonist.

Inhibition of TLR9 in rats with PIA reduced inflammation and erosion whereas stimulation of TLR7 aggravated the disease. These results suggest different roles for TLR7 and 9 in the initiation phase of PIA and thus an important involvement of the DNA recognizing TLR9 and the RNA recognizing TLR7 in the initiation of autoimmune arthritis which needs to be further elucidated.

**70 MAZR - a comprehensive analysis of its expression and function in CD4+ helper T cells**

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T cell development and function are tightly regulated by transcriptional and epigenetic mechanisms. We previously demonstrated that MAZR, a BTB-domain containing zinc finger transcription factor (also known as Patz1), is a regulator of CD8 expression and also that MAZR is part of the transcription factor network regulating CD4/CD8 lineage differentiation of double-positive (DP) thymocytes. MAZR is highly expressed in double-negative (DN) and DP thymocytes and down-regulated in single-positive (SP) thymocytes. This might suggest that the down-regulation of MAZR is essential for the generation of SP cells. However, it is not known whether MAZR expression is modulated during peripheral CD4+ T cell subset differentiation and whether enforced expression of MAZR and thus high MAZR expression levels would interfere with T helper function. To address these issues, we are performing a detailed expression analysis of MAZR in peripheral T helper subsets using quantitative real-time and immunoblotting approaches. Moreover, we are performing gain-of-function studies using retroviral-mediated overexpression strategies to test whether high MAZR levels impair the differentiation of CD4+ T cells. Results from our ongoing experiments will be presented.

**71 The histone acetyl transferase GCN5 controls virulence of candida albicans through regulation of multiple pathways**

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The increased incidence of invasive fungal infections has changed the landscape of fungal infections worldwide and greatly impacts healthcare management. Candida albicans is the most prevalent opportunistic human fungal pathogen and the leading cause of Candida blood-stream infections. In addition to the known pathogenicity mechanisms, epigenetic reprogramming has recently emerged as a prime mechanism critically determining the outcome of host-pathogen interactions. Acetylation and deacetylation of chromatin constituents by histone acetyltransferases (HATs) and histone deacetylases
(HDACs) are the two dynamic post-translational modifications modulating gene expression and pathogenicity. In fact, targeting HATs and HDACs could constitute a novel therapeutic approach to treat fungal infections. However, the molecular mechanisms of HATs and HDACs in fungal pathogenesis remain to be further explored. Here, we present novel data on the first phenotypic and molecular characterization of GCN5, a prototypic HAT in C. albicans. Lack of GCN5 leads to pronounced azole resistance, cell wall stress susceptibility, as well as cell morphology defects affecting filamentation and virulence. Surprisingly, gcn5 deletion mutants also displayed constitutive hyper-activation of the Cek1 and Hog1 kinases, both operating in fungal Mitogen-Activated Protein (MAP) kinase signaling pathways essential for virulence. Remarkably, the absence of Gcn5 dramatically debilitates acetylation of H3 and H4 at certain lysine residues, implying that the target genes are essential in controlling virulence in response to host signals. We are now using a proteomics approach to identify the Gcn5 interaction partners but also the associated acetylome. Our results suggest that the Gcn5 HAT may act as a molecular switch controlling the cross-talk of different signaling pathways to control fungal virulence.

Bartonella species - another tick-borne pathogen?

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Bartonella species are aerobic, Gram negative, facultative intracellular bacteria, which cause a variety of human and non-human diseases. Until now 24 Bartonella species and 3 subspecies have been described including at least 12, pathogenic for humans. In this study 4 strains of Bartonella (B. clarridgeiae, B. grahamii, B. henselae, and B. doshiae) were cultivated and used as positive controls for the design of a Bartonella specific PCR. Due to the fact that Bartonella species are fastidious bacteria growth studies with different media have been performed. Two different PCRs were established: a Bartonella specific one and one for species differentiation. Therefore three target sequences were chosen: the 16S rRNA gen, the 16-23S intergenic spacer region and the riboflavin synthase gen, whereas the primers for the ITS region were used for species differentiation. With these Bartonella PCRs a tick library was screened. The tick library consists of 10.326 Ixodes ricinus ticks (adult, nymphs and larvae) collected at different locations in Austria. This screening was performed with two different PCR assays: a Real Time PCR and a Nested PCR. The results and the two analysing methods were compared to each other. Further, two libraries of human sera were screened for IgG-antibodies to B. henselae and B. quintana by an immuno fluorescence assay (IIF-Test, Euroimmun AG). One serum library consisted of sera from hunters who are regularly tick exposed, and the other serum library comprised sera from blood donors. Both groups of persons were from an almost matching geographical area. The serum samples were obtained from the serum collection of the Institute for Hygiene and Applied Immunology. The results of serological testing were that 6% of the donor sera and 2% of the hunter sera were IgG seropositive for B. henselae, but 22% and 23% were IgG seropositive for B. quintana, respectively.

A newly established real-time PCR for detection of Borrelia miyamotoi in Ixodes ricinus ticks
Borrelia miyamotoi is a relapsing fever spirochete that was first discovered in Japan in 1995. Since then it has been found in other parts of the world, including the United States and Eurasia. Recently, evidence rendering B. miyamotoi as a causative agent of human disease is accumulating with increasing pace. We report the detection of B. miyamotoi in Ixodes ricinus ticks from Austria for the first time. A total of 350 ticks collected in all federal states of Austria were analyzed for the presence of DNA sequences of B. miyamotoi. Three ticks gave positive results in a nested PCR specifically targeting the B. miyamotoi glycerophosphodiester phosphodiesterase gene (glpQ). These ticks were found in Tyrol (n=2) and Lower Austria (n=1). Results were confirmed by sequencing the amplified glpQ gene from the positive isolates. Additionally we characterized the isolates by sequencing the 16S rRNA gene as well as the 16S-23S intergenic spacer (IGS). Comparison of the IGS with sequences deposited in GenBank revealed a 97% identity to B. miyamotoi FR64b, a Japanese strain, whereas being one hundred percent identical to a Swedish isolate. Analysis of the 16S rRNA gene sequence showed highest similarity to a B. miyamotoi isolate originally obtained from the Czech Republic. Moreover, in order to speed up future sample processing, we developed a robust Real-Time PCR assay which unambiguously detected B. miyamotoi in all ticks initially tested to be positive. Our results consolidate the picture of a European-wide distribution of B. miyamotoi and once more underscore the need for clinical awareness to clarify possible involvement of this species in human disease.

Role of N-Myristoyltransferase family members in the covalent modification of Enterovirus proteins

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The family of picornaviridae comprises many important human and animal pathogens such as polio-, rhino-, and coxsackie- virus. Notably, in most of these non-enveloped icosahedral (+)ssRNA-containing viruses the N-terminus of the capsid protein VP4 is myristoylated, with a still incompletely understood function in the virus life cycle. N-myristoylation is a co-translational modification where a 14 carbon long saturated fatty acid (myristate) is covalently linked to the alpha-amino group of a N-terminal glycine, catalyzed by the enzyme myristoyl-CoA:protein N-myristoyltransferase (NMT). Many mammalian, fungal, and viral proteins are myristoylated and this lipid modification has been proven essential for their proper localization and/or biological activity. In mammals, two isozymes (NMT1 and NMT2) are expressed from different genes, with only partially overlapping function. We now demonstrate their differential importance in the replication of picornaviruses by focusing on Coxsackieviruses 3B (CV3B), a major cause of viral myocarditis. Infection of human haploid cell lines Hap1 NMT1Null and Hap1 NMT2Null, which do not
express the respective NMT isozyme, with an eGFP-expressing CVB3 resulted in reduction of cell to cell spreading and viral titers in comparison to wild type Hap1. As the observed effect was more prominent for NMT1 enzyme, it likely implicates this isozyme as the major NMT in myristoylation of VP4. To facilitate elucidation of the role(s) of this lipid moiety we currently attempt incorporation of a myristoyl-analog into CVB3 to enable subsequent bioorthogonal “click-chemistry”-based tagging, for which we outline the procedure and present first data. Lastly, exploiting the novel bacterial demyristoylase IpaJ in combination with a mass spectrometry calibrated reversed-phase chromatography we highlight its future usefulness to evaluate the importance of the myristoylated VP4 in the penetration of the host cell.

75 Binding of tick-borne encephalitis virus to cells
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Introduction: The major surface protein E of flaviviruses is crucial for cell entry and mediates both, binding to a cellular receptor as well as fusion of the viral and endosomal membrane after uptake by receptor-mediated endocytosis. The E protein forms an icosahedral lattice of 90 homodimers at the virion surface. Recent evidence has indicated that E is subject to dynamic movements leading to the transient exposure of structures that would be buried in a rigid viral envelope. Flaviviruses infect a wide variety of cell lines from different host species and several attachment factors have been identified, but a high affinity receptor has not been described yet. Objectives: In this study, we want to investigate the binding of tick-borne encephalitis virus (TBEV) to cells and identify factors that could influence this process. Moreover, we will assess the role of the dynamic TBEV surface in binding and its impact on infectivity. Material and Methods: We used well-characterized TBEV preparations and different cell lines for our study. Bound and unbound virus was quantified by qPCR and infectivity was measured by focus forming assays. Human rhinovirus 2 (HRV2), with a known high-affinity receptor, served as a control. Results and Conclusion: TBEV bound very inefficiently to BHK-21, Vero, CHO-K1, pgsA-745 and HeLa H1 cells with only 0.2 – 0.7% of input virus attached to cells. In agreement with these data, the infectious unit-to-particle ratio was approximately 1:350 to 1:1100, depending on the cell lines used. Currently we investigate factors that underlie these phenomena. In addition, we analyze different conditions that could potentially enhance binding of TBEV to cells.

76 Flavivirus E protein stem interactions in virus entry
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Introduction: Flaviviruses enter cells by receptor-mediated endocytosis. After virus uptake, fusion of the viral and the endosomal membrane is triggered by the slightly acidic pH in endosomes and mediated by the viral envelope protein E. The current fusion model is based on atomic structures of truncated forms of the E protein in their dimeric pre- and trimeric post-fusion conformation. These structures lack the two transmembrane-domains and the so-called “stem”-region. The stem connects the ectodomain and the membrane anchor and is hypothesized to be essential for fusion by “zippering” along the trimer core during the conformational changes of E. Objectives: Since stem interactions are essential in providing energy for fusion, we want to gain information on these interactions as well as their role in the fusion process by a mutagenesis approach using tick-borne encephalitis virus (TBEV), a major human pathogenic flavivirus. Materials and Methods: We introduced modifications (point mutations, deletions) into the stem of recombinant E proteins as well as an infectious clone of TBEV and analyzed their effect on E protein trimerization, trimer stability and infectivity. Results and Conclusion: We identified important interaction sites between the stem and the trimer core involved in the stabilization of the post-fusion conformation. In addition, replacing conserved residues in the stem led to a strong reduction in the production of infectious particles. Currently, we investigate whether the observed phenotypes are caused by defects in entry, assembly or both processes.

77 Neutrophil responses to adjuvants used in allergy vaccines


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Most subcutaneous allergy vaccines in Europe contain alum as adjuvant, and a few monophosphoryl lipid-A (MPL), a TLR-4 agonist. For alum it has been shown in mice that neutrophil-derived DNA mediates adjuvant activity. Neutrophils are the most abundant white blood cell population and part of the innate immune system. As a first line of immune defense their repertoire includes the ability to trap, kill and phagocytose pathogens extracellularly by releasing DNA and granular material, so-called neutrophil extracellular traps (NETs). We intend to investigate the response of human neutrophils to alum and MPL and their possible role in the immune response induced by allergen-specific immunotherapy. To this aim freshly isolated human neutrophils are seeded on coverslips and stimulated with NET-inducing factors including PMA and LPS in comparison to alum and MPL. Formation of NETs is evaluated by staining of DNA or granular proteins and fluorescence microscopy. Our first experiments showed that the two adjuvants elicit different kinds of NET responses. The response to MPL showed expected similarity to the LPS-triggered NET-formation with single DNA-filaments, granular myeloperoxidase sticking to them and intact nuclei. In contrast, alum-triggered NETs containing myeloperoxidase were observed in immense clusters, which were also associated with vital nuclei. None of the adjuvants caused cell death, as it is observed with PMA. Our preliminary results by fluorescence analysis suggest a much stronger NET response to alum than to MPL or LPS. However, for quantitative statements the amount of released DNA and or neutrophil elastase has to be determined.
Is immune responsiveness to routine vaccines influenced by allergy and allergen-specific immunotherapy? - A clinical trial


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Type I allergies have increased drastically and afflict up to 30% of western populations. Allergic sensitization results in Th2 biased immune responses and specific immunotherapy (SIT) leads to immuno-modulation via IL10/TGF and shift to a Th1 profile (IFNγ). In a clinical trial we investigate whether responsiveness to routine vaccines is altered by allergy: allergic patients, either symptomatically treated or undergoing SIT and healthy controls receive a booster vaccination against tick-borne encephalitis (TBE). To test primary responsiveness, hepatitis A vaccination is additionally given to a subgroup of hepatitis A seronegative individuals. Immune responses to vaccination are evaluated via specific Ab-titers, cytokine production of re-stimulated PBMC and quantification of naive, memory, and regulatory subsets of B- and T-lymphocytes. Our results to date show that humoral responses to booster vaccination are not reduced in allergic patients +/-SIT. Cytokine production in-vitro (IFNγ, IL2, IL10) appears to not correlate with Ab-titers and no baseline IL10 is detected in allergic +SIT. Considerable variation of naive and memory subsets as well as differentiation status of CD4 and CD8 T-cells are observed in both allergic and allergic +SIT, e. g. expanded CD8 T-EMRA compartment and increased late differentiated CD8 and CD4-memory T-cells. Also an increased % of FOXP3+ T-regs is present in allergic +SIT. Further analyses of Ab-titers, quantification of B- and T-cell sub-populations as well as cytokine production in re-stimulated PBMC is ongoing and additionally the allergic- and SIT-status of participants will be correlated with humoral/cellular immune responses. The results of this study will complete our understanding of vaccine responsiveness in these patient groups and eventually clarify whether allergic sensitization and SIT in particular lead to altered/impaired vaccine responsiveness and demand adaptations of vaccination schedules.

Investigating the interactions between the picornaviral proteases and host factors eIF4GII and eIF4E

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Successful viral replication requires rapid modulation of the infected cells physiology. Interference with the host cells machinery for protein synthesis is a mechanism favored by positive strand RNA viruses, such as poliovirus, human rhinovirus (HRV) and foot-and-mouth disease virus (FMDV), to increase the efficiency of their translation. eIF4G cleavage is performed by the FMDV leader protease (Lb\textsuperscript{pro}) or the 2A\textsuperscript{pro} of HRV or PV. Picornaviral cleavage of eIF4G is a determinant of virulence, but has not yet been elucidated structurally. We are therefore investigating how the picornaviral proteases interact with eIF4G. In our experiments, we use an eIF4GII fragment consisting of residues 550-745 featuring the conserved eIF4E binding motif and the cleavage and binding site of the Lb\textsuperscript{pro}. We showed that eIF4E enhances the cleavage of eIF4G by the Lb\textsuperscript{pro} or the 2A\textsuperscript{pro} in an in vitro assay. A stable complex of eIF4G and Lb\textsuperscript{pro} or the 2A\textsuperscript{pro} is not formed in the absence of eIF4E. However, when eIF4E is present, a heterotrimeric complex is stably formed. Analysis of the ternary complex of eIF4G/eIF4E with a catalytically inactive Lb\textsuperscript{pro} showed that the stoichiometry in molar terms was 1:1:1. ITC experiments showed results and confirmed that the dissociation constant of the eIF4G/eIF4E complex with the Lb\textsuperscript{pro} is about 100 fold lower than that for each single protein to the Lb\textsuperscript{pro}. Using triple resonance NMR experiments, we could assign about 160 out of 196 residues of a \textsuperscript{13}C/\textsuperscript{15}N labelled eIF4GII\textsubscript{550-745}. In addition, complex formation between a \textsuperscript{15}N labelled eIF4G, eIF4E and an inactive Lb\textsuperscript{pro} could be observed by NMR and showed that the \textsuperscript{15}N signals for at least 30 residues were shifted. Moreover, we identified residues (C133, Q185R, E186K) on the Lb\textsuperscript{pro} which interfere with binding of the eIF4G/eIF4E complex and reduce the cleavage of full length eIF4G/eIF4GII.

80 Alveolar macrophage derived type I interferon directly protects alveolar epithelial type II cells from cell death in a murine pneumonia model

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Streptococcus pneumoniae are Gram-positive bacteria that colonize the lung and can lead to severe pneumonia. Several bacterial components locally trigger a massive inflammatory response after contact with alveolar immune cells and epithelium which rapidly escalates into systemic inflammation and life-threatening sepsis. In this study we focus on the role of type I interferon, a cytokine classically associated with viral infections and autoimmunity, in pneumococcal pneumonia. It has been shown that Streptococci among other bacteria can induce this group of cytokines in various cell types in vitro via different mechanisms, but the biological relevance of these findings is still unclear. Thus, we wanted to study its potential as immune-modulatory cytokine to protect from fatal outcome of pneumonia. Our results suggest that type I interferon is preventing tissue damage in the lung as well as systemic dissemination of bacteria and that those effects are dependent on bacterial DNA. Further we found type
I interferon induced in alveolar macrophages after S. pneumoniae infection in vivo. By using lung cell specific conditional type I interferon receptor knock-out mice we could show that type I interferon directly acts on alveolar epithelial type II cells and prevents them from cell death during infection as well as sterile lung injury. In this study we could describe for the first time which cell types of the alveolus produce and respond to type I interferon in a model of murine pneumonia. Further, we want to elucidate the molecular mechanism by which type I interferon acts on alveolar epithelial cells type II.

81 The non-receptor tyrosine kinase Tec regulates the noncanonical caspase-8 inflammasome in antifungal immune responses


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Tec family kinases are intracellular non-receptor tyrosine kinases implicated in numerous cellular functions, including B-cell and T-cell regulation. However, a role in microbial pathogenesis has not been described yet. Here, we identified Tec kinase as a key mediator of the inflammatory signaling response in bone marrow macrophages invaded by the human fungal pathogen C. albicans. Tec is required for both activation and assembly of the noncanonical caspase-8, but not of the caspase-1 inflammasome, during infections with fungal but not bacterial pathogens, ultimately triggering the antifungal response through IL-1[beta]. Furthermore, we identified dectin-1 being required for Syk-dependent Tec activation and confirm that activation of the caspase-8 inflammasome is dependent on fungal [beta]-glucan exposure. Hence, Tec represents a novel innate-specific inflammatory kinase, whose genetic ablation or inhibition by small molecule drugs strongly protects mice from fungal sepsis. Our data demonstrate a therapeutic potential for Tec kinase inhibition to combat invasive microbial infections by attenuating the host inflammatory response.

82 Systems response and resolution after S.pneumoniae infection


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The Gram-positive bacterium Streptococcus pneumoniae (S. pneumoniae) is the leading causative pathogen of community-acquired pneumonia (CAP). S. pneumoniae as a natural commensal of the upper respiratory tract can overcome structural barriers, enter the terminal airways and elicit an innate immune response that normally serves to eliminate this pathogen. This recognition/response phase has been well studied. However, effects of this pathogen on distant organs, the ensuing systemic inflammatory response and how this response is fine-tuned and ultimately resolved is poorly understood. Indeed, exaggerated inflammation caused by an impaired resolution can be associated with immunopathology and death. Here, using systems wide approaches that encompass RNA Seq and proteomics, we aim to identify systemic changes during pneumonia as well as novel homeostasis-promoting factors. Mice were infected with S. pneumoniae and samples from the lung and distant organs were taken at different timepoints for RNA Seq, proteomics and bacterial quantification. Early inflammatory markers, including neutrophil influx, were observed 4-6h post infection, when clinical signs of disease were undetectable and bacteria were present only at the primary site of infection. Later (>48h post infection), bacterial dissemination into the blood and distant organs occurs, leading to systemic tissue damage and organ failure. RNA sequencing not only revealed well described key mediators of inflammation but also potential novel regulators of host immunity during pneumococcal pneumonia. Future work will involve quantitative proteomics to validate the observed transcriptional changes. This project aims to utilize systems wide approaches to understand the systemic responses and return to homeostasis during pneumococcal pneumonia, a major cause of morbidity and mortality worldwide.

83 Imaging Antigen Recognition of Human T-cells in Health and Disease

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Autoantigen-specific T cells frequently escape negative selection in the thymus and circulate in the periphery without posing any harm. However, such T cells have in essence the potential to become activated and cause autoimmunity. In particular, high affinities of the T cell receptor (TCR) for its antigen, i.e. peptide loaded MHC (pMHC), have been hypothesized to promote the development of autoreactive T cells and the onset of disease.

To test this hypothesis in a systematic fashion we will isolate (auto)antigen-specific T-cells with the use of properly refolded, assembled and fluorescence-labeled multimeric pMHC complexes from peripheral blood of healthy and Type 1 diabetes (T1D) donors. We will next analyze these T-cells with regard to their TCR-pMHC affinity and correlate measured affinities with differentiation state, antigen sensitivity and the propensity to form mature immunological synapses on functionalized planar supported lipid bilayers (SLBs). As a proof of principle for method development, we have successfully generated HLA-
A*02/cytomegalovirus phosphoprotein 65 (CMVpp65) streptamers for the isolation of HLA-A*02-positive, CMVpp65-specific T cells. We have also established an SLB system, which features human ICAM-1, B7-1 and appropriate pMHCs and which allows high resolution imaging of synapse formation by streptamer-isolated human T-cells. Furthermore, we are devising a quantitative assay to determine synaptic TCR-ligand affinities and the rate of TCR-ligand unbinding on a single cell level. We are now extending our overall approach to comparing autoantigen-specific T cells in healthy and T1D individuals.

84 Recombinant production of the major peanut allergen Ara h 2 in insect cells

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Background: Natural (n) Ara h 2 is difficult to purify free of contaminating Ara h 6 which can distort immunological characterizations of Ara h 2. Recombinant protein production offers an alternative to obtain pure Ara h 2. The optimal expression system has to be selected according to the characteristics of the desired protein. In this study, the baculovirus expression system was selected for recombinant (r) Ara h 2 production. Methods: A synthetic Ara h 2 gene with a hexahistidyl or a strep tag was inserted into the pACEBac vector. Following propagation in E. coli DH10MultiBacY cells were used to generate a recombinant bacmid vector. The recombinant bacmid DNA was then used to transfect Sfodoptera frugiperda 9 (Sf9) cells to generate recombinant baculovirus. For recombinant protein expression, Sf 9 and Trichoplusia ni BTI-TN5B1-4 “High Five” (Hi5) cells were infected with a multiplicity of infection (MOI) of 5. To evaluate the expression of Ara h 2, SDS-PAGE analysis and Western blots with supernatants and cell pellets were carried out.

Results: Western blots incubated with either anti-his or anti-strep antibodies or sera of Ara h 2 sensitized patients revealed approximately 20 kDa bands in cell pellets and supernatants. This strongly indicates a successful expression of rAra h 2 in the baculovirus expression system.

Conclusion and Outlook: In the next steps, purified rAra h 2 will be compared with nAra h 2 using ELISA, RBL- and BAT-assays. If both proteins show similar results, nAra h 2 could be replaced by rAra h 2 eliminating the problems encountered when using nAra h 2 extracts.

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85 High resolution imaging to quantitate the molecular dynamics of antigen recognition by cytolytic T cells
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T-cells are exquisitely sensitive towards antigen as they can detect with their T-cell antigen receptors (TCRs) the presence of even a single antigenic peptide-loaded MHC molecule (pMHC) among thousands of structurally related yet non-stimulatory pMHCs. This is despite the rather moderate affinity between TCRs and antigenic pMHCs (1-100 µM). At current it is not clear how T-cells achieve such sensitivity, even though much effort has been invested in identifying and characterizing the components of the recognition and signaling machinery involved. One particular stepping-stone is the fact that antigen recognition takes place within the special constrains of the immunological synapse, the area of contact between the T-cell and its antigen-presenting cell (APC). Here receptors and their ligands are pre-oriented, possibly clustered in specific membrane domains and subjected to cellular forces. Since biochemical experimentation invariably requires the destruction of at least one of the synaptic membranes it does not account for the specific microenvironment in which T-cell antigen recognition occurs. We are therefore employing a high resolution imaging approach in which we confront cytolytic TCR transgenic T-cells with a glass-supported lipid bilayer (SLB) functionalized with the adhesion molecule ICAM-1, the co-stimulatory molecule B7-1 and nominal pMHCs. This system allows us to monitor TCR-pMHC binding events in a synaptic environment through Förster Resonance Energy Transfer (FRET) measurements. Furthermore ensuing signaling consequences can be simultaneously tracked with the use of ectopically expressed GFP-fusion reporters. In particular, we would like to reveal the molecular involvement of the CD8 co-receptor known to boost T-cell antigen sensitivity by a factor of 10 to 50. Moreover, we intend to uncover the influence of galectins on antigen recognition, which bind post-translationally modified glycoproteins, such as the TCR and many accessory proteins, and which are differentially expressed during T-cell development.

86 The role and mechanism of CD40/CD40L expression on different T-cell subsets

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Background: Several T-cell costimulatory pathways have been identified that play a critical role in promoting transplant rejection. Their function on distinct T cell subsets, however, remains incompletely defined. Therefore we aim to investigate the role of CD40 and CD40L in specific T cell subsets, in particular in conventional T cells versus regulatory T cells. Methods: The expression of CD40 and CD40L was analyzed on T-cell subsets in vitro. Therefore B6 splenocytes were cultured under different experimental conditions, with αCD3/αCD28 and PMA/ionomycin being used as stimulators. The
expression of surface CD40L and preformed CD40L within lysosomes was analyzed with a mobilization assay by capturing and stabilizing the protein that has been delivered to the cell surface during 2-48h of incubation. CD40+/− and CD40L+/− T cells were further analyzed by FACS for their different expression behavior of selected markers. Results: Preliminary protein kinetic studies demonstrate that CD40L is transiently and inducible expressed in both CD3+CD4 (85%) and CD8 (29%) subsets with maximum expression after 6 hours of PMA/I activation. Additionally we found that ionomycin alone strongly triggers CD40L expression in CD3+CD4+ (85%), whereas less so in CD8+ (3%) cells. Notably, 40-50% of CD3+CD4+Foxp3+ cells expressed CD40L after 6h αCD3/αCD28 or PMA/I activation. While Helios and CD62L were expressed more frequently within Foxp3+ CD40L negative cells (Helios CD40L− 72%, CD40L+ 44.6%; CD62L CD40L− 22%, CD40L+ 10.6%), ICOS expression was higher on CD40L+ (36.4%) than CD40L− (28%). Only a small subset of CD4 and CD8 T-cells (~1%) expressed CD40 and within those around 7-8% were single positive for CCR6, 13-24% for CXCR3 and around 67-76% double negative for both. Conclusion: This preliminary in vitro study reveals a time and stimulus-dependent induction of CD40L on distinct T-cell subsets, including FoxP3 Tregs, whose functional relevance is investigated in ongoing studies.

87  Quantifying antigen thresholds for CAR T cell mediated tumor cell killing

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T cells engineered to express chimeric antigen receptor (CARs) directed against tumor-associated antigens have shown great promise for cancer immunotherapy. A distinct advantage of CAR-driven target recognition is the HLA-independent mode of antigen binding, which is encoded by the specificity of the single chain antibody fragment (scFV), the functional ligand-binding unit of the CAR. Consequently CAR T cells can in principle be engineered to target any given tumor-specific antigen. Many aspects of CAR-T cell tumor recognition are however not well understood which complicates the rational optimization of CARs. Of note, CAR T cell antigen sensitivity is generally estimated to be orders of magnitudes lower than that of T cells specific for MHC-bound antigens. This is despite CARs featuring a significantly higher affinity for antigens than TCRs for peptide antigen-loaded MHCs. In order to facilitate rational CAR optimization we wish to establish a cell-based assay system, which allows precise quantitation of CAR-T cell antigen sensitivity. To this end we ectopically express a membrane-bound version of single chain version of avidin, which is monovalent for biotin binding and which serves to attach defined quantities of site-specifically biotinylated and fluorescence labeled antigens of choice, such as CD19 and ROR1, for CAR T cell recognition. Importantly, the complexity of the three-dimensional architecture of target cell membrane, which is known to affect T cell antigen sensitivity, remains
conserved. We expect to be able to precisely determine the antigen thresholds per cell required for CAR T cell mediated killing by flow cytometry. Furthermore we intend to apply quantitative (single molecule) fluorescence microscopy to determine the number of antigens required in situ for synapse formation, CAR T cell activation and killing. We will use this system to screen novel CAR designs for function.

88 Investigating the immunomodulatory potency of stress hormones interacting with molecular allergens

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Allergic disorders result of a deviant immune response toward innocuous environmental substances, resulting in a so-called Th2 immune response and the production of allergen specific IgE. A second allergen contact initiates the effector phase, with a cascade of molecular and cellular events leading to acute inflammation. There is a growing body of evidence that catecholamines might induce a Th2 shift and it is well known that stress may exacerbate asthma and atopic dermatitis. Herein we propose that exogenous allergens and endogenous stress molecules may interact with each other, thereby synergistically acting on immune deviation towards allergy. Our publication showed striking structural homology between Bet v 1, the major allergen from pollen of white birch (Betula verrucosa) and human lipocalin-2 (LCN2). Like LCN2, Bet v 1 has a molecular pocket where it binds complexes of iron and siderophore. Depending on the pocket load, Bet v 1 and other lipocalins are able to modulate immune response. Adrenaline is a naturally occurring Fe-binding molecule and therefore functions as a siderophore. Interestingly, our in silico data indicate a strong affinity of Fe(III) adrenaline complex for Bet v 1 (24 nM). We will systematically address this question by i) quantifying this interaction using radioactive and/or fluorescence titration ii) investigating immunological properties of Bet v 1 or LCN2/adrenaline:iron complexes on mediator and cytokine secretion in PBMC-derived macrophages, various immune cell lines and ex-vivo model (primary mast cells isolated from nasal tissue explants) and iii) bringing the in vivo proof of concept using mouse model to investigate the effect of adrenaline on the threshold for allergen challenges during the effector phase of IgE-mediated allergy. By analyzing the
interplay of adrenalin:iron with Bet v 1 or its endogenous counterpart LCN2 we aim to reveal the complex mechanisms behind the effect of stress hormones accelerating allergy.

89  **Solute carriers: Proteins at the interface of host metabolism and viral life cycle**

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Host factor requirements for many classes of viruses are yet to be unraveled. Replication of the viral genome and synthesis of viral proteins inside the host cell are associated with altered, often enhanced cellular metabolism and increased demand in nutrients and specific molecules. With some 400 identified members in humans, the solute carriers (SLC) represent the largest family of trans-membrane proteins dedicated to the transport of small molecules, such as amino acids, sugars, nucleotides and ions. Herein we aim to characterize the role of host SLCs in viral replication as well as confirm their function as new regulatory group of proteins in the antiviral immune response. Upon integration of the multiple large-scale datasets from recent genome-wide screens, a group of around 20 SLC proteins has been identified to have a function linked to viral replication or immune response. We systematically inactivated these genes in the HAP1 cell line using the CRISPR-Cas9 system. A primary screen performed using the Influenza A/WSN/33 strain suggests that mutations in several of the SLC genes from our pool substantially affect the susceptibility of these cells to infection. We plan to carry on further characterization of the most interesting SLC candidates in order to dissect their role in the viral life cycle. Moreover, we will study the protein-protein interactions of their gene products and will try to identify their natural cargo that may be critical during the viral life-cycle. Together, this “viral transportome” may offer new insights into possible strategies to pharmacologically interfere with viral infections.

90  **Lymphocyte-specific protein tyrosine kinase Lck in the process of T-cell-activation**

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It is well known that lymphocyte-specific protein tyrosine kinase (Lck) plays a major role in the process of T-cell activation by antigen-presenting cells (APC). Upon activation, Lck phosphorylates the CD3 chains of the T cell antigen receptor (TCR) and in addition zeta-protein-associated protein kinase (ZAP-70), which powers on several proteins most importantly PI3K and phospholipase C (PLC). As consequence, the activation of these molecules leads to the opening of endoplasmic reticulum and membrane-bound
Ca2+ channels and thereby changes the gene expression pattern to transform T-cells into an activated state. How exactly the mechanism behind Lck and T-cell activation functions is still not entirely understood and therefore aim of this project. Of special interest is the context of how lipid-drugs such as statins and unsaturated fatty acids affect T-cell membrane composition and Lck activation. This would be as interesting as relevant for cardiovascular disease treatment and tumor progression.

91 Proteomic investigation of the ITK signaling cascade

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Idiopathic CD4+ lymphopenias constitute a heterogeneous group of primary immunodeficiencies with mostly unknown genetic etiology. It has been reported that patients with biallelic germline mutations affecting interleukin-2-inducible T-cell kinase (ITK) may present with predominant CD4 lymphopenia and absence of NKT-cells. However, the mechanism is still only poorly understood. A novel phosphorylation target of ITK, namely interferon regulatory factor 4 (IRF4) binding protein (IBP), has been described recently. IBP is a guanine nucleotide exchange factor with implemented roles downstream of the T cell receptor complex. Interestingly, IBP has been shown to interact with IRF4, a transcriptional activator, and Irf4-/- mice also display reduced numbers of iNKT cells. We sought to elucidate the signalling cascade of ITK, IBP and IRF4 by identifying interaction partners which should help to understand the mechanisms of iNKT cell development. To this end the baits ITK, IBP and IRF4 were Strep-HA tagged and transfected in Hek FlpIn cells as an inducible model cell line. Protein purification was carried out according to standard operation protocol of the CeMM mass spectrometry facility, baits and interactors were tryptically digested and further submitted to the facility for analysis. Interaction partners of ITK, IBP and IRF4 could be identified and will be validated. This will help us to have insight in the potential pathway of iNKT cell development and broaden our knowledge on ITK biology.

92 Characterization of the CD8+ T cell response to allergens

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Allergy is the abnormal immune response against harmless antigens. Like any immune response, allergic reactions are mediated by both innate and adaptive immunity. T cells are part of adaptive immunity and play a main role in the development of type I allergy. The function of CD4+T cells in the pathogenesis and maintenance of allergic disorders have been broadly investigated while the role of CD8+T cells is poorly
understood. Previous studies have demonstrated a controversial function of CD8+T cells in allergic disorders in human and murine models. The aim of this project is to identify and characterize allergen-specific CD8+ T cells in patients with different manifestations (rhinoconjunctivitis, atopic dermatitis and atopic bronchial asthma) of type I allergy. Different seasonal and perennial allergens will be studied. Allergen specific T cells from PBMCs will be expanded in vitro and these T cell lines and cloned T cells will be characterized for their phenotype and function. By now, we have optimized the expansion of allergen-activated CD8. Peripheral blood from patients were stained by proliferation dyes and stimulated with allergen. Proliferating cells were expanded by addition of growths factor/s in short time cultures and characterized. We could find significant CD8+T cell proliferation in all allergic diseases, but larger numbers of allergen-reactive CD8+T cells were found in T cell lines induced by house dust mite & grass pollen extracts compared to pollen extract birch. Next step is to identify the functionality of these allergen-reactive CD8+ T cells.

93 Innate lymphoid cells type-2 contribute to macrophage polarization and reduce defenses against pneumococci

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Streptococcus pneumoniae is the leading cause of community-acquired pneumonia (CAP), a major cause of death in western countries. An alternative polarization of alveolar macrophages (AMs) is detrimental in pneumonia and can be induced by IL-13 during asthma, a risk factor for CAP. We hypothesized that pulmonary IL-13 might contribute to an alternative polarization of AMs, enhancing the susceptibility to pneumococcal pneumonia. Il-13/-/ mice challenged with S. pneumoniae exhibited earlier neutrophil influx and improved bacterial clearance compared to their WT counterparts. In il-13/-/ mice the AMs show a pro-inflammatory phenotype and increased CXCL1 secretion in vitro. Lung innate lymphoid cells type II (ILC2s) can secrete IL-13 in response of IL-33 released in case of cell damage. With the use of the il-13-Tomato reporter mice and intracellular staining for IL-13, we demonstrated that ILC2s represent a unique and constant source of IL-13, in an IL-33 dependent manner. Mice deficient in ILC2s recapitulated the phenotype of il-13/-/ mice. This study importantly discloses that lung ILC2s contribute to the alternative polarization of AMs at the expense of defenses against pneumococcus.
Non-invasive tissue specific reporter system for monitoring of skeletal muscle cell differentiation

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Detection of skeletal muscle differentiation plays a key role for tissue engineering. In this study, we developed a reporter system to investigate the effectiveness of murine skeletal muscle differentiation. The use of a tissue specific promoter combined with a non-invasive bioluminescence assay allowed the evaluation of biological processes during myogenic differentiation in 2D and 3D culture systems as well as in bioreactors. To construct the muscle creatine kinase (MCK) specific reporter vector (pE3MCK-MetLuc ± CMVe), triple tandem of MCK enhancer was positioned to its truncated basal promoter (87bp) upstream of either a secreted luciferase or a fluorescent protein. In addition, up to three different myogenesis related miRNA seed sequences were ligated into 3'UTR of DNA, in order to develop tracking strategy by reducing background signals in non-muscle cells. Cells were transfected with indicated constructs by using lipofection. The reporter gene signal in the supernatant was either detected using a luminometer after conversion of substrate into light units (secreted luciferase) or by fluorescence microscopy (fluorescence protein). The constructs exhibited strong luciferase and fluorescence expression in cells undergoing myogenic differentiation compared to non-muscle cells. Reconstruction of pE3MCK-MetLuc vector by ligating of miRNA seed sequences resulted in low background signals in non-muscle cells. Tissue-specific promoters combined with E-boxes and miRNA seed sequences allow the amplification of tissue specific signals as well as kinetic monitoring of myogenic differentiation in a nonsample-destructive manner.

The impact of phosphatidylinositol-4,5-bisphosphate (PIP2) on serotonin transport function

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Lipid oxidation patterns and kinetics under senescence-promoting stress in keratinocytes

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Oxidation of lipids and proteins is not only a manifestation of aged skin but also potentially causative for age-related aesthetic decline and pathologic damage. Extrinsic oxidative stress promotes the accumulation of reactive lipid oxidation products. Polyunsaturated fatty acids of phospholipids (PL) are easily oxidized by extrinsic stressors that promote skin aging, and the resulting lipid mediators elicit stress responses. To study in keratinocytes, which oxidation products are generated upon replicative senescence and environmental UV stress and to study the kinetics of intrinsically generated and extrinsically added oxidized PL, we performed lipidomic analysis. We applied a HPLC-tandem-MS method recently developed by us and quantified over 500 PUFA-PL oxidation products in Keratinocytes immediately and 24 hours after irradiation with 40J/cm² UVA-1. We found that immediately after UVA-1 radiation PL containing esterified dicarboxylic acids show higher accumulation than PL hydroperoxides and –hydroxides. Levels of dicarboxylic acid containing PL returned to baseline after 24h. Exogenously added UV-oxidized PL initially underwent rapid oxidation and chain shortening, whereas after 24 hours a massive increase of F and E,I,D class PL-isoprostanes was detected. We found also that PL containing esterified dicarboxylic acids and isoprostanoid PL accumulate during replicative senescence of keratinocytes. As isoprostanes and isoprostane containing PL are correlated to aging (also of the skin), the modulation of isoprostane levels by UVA may be a novel mechanism contributing to photoaging.

97 Communication in the DNA-damage response

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The DNA-damage response [DDR] is composed of a complex signaling network that guards the integrity of the genome and thus forms an indispensable barrier in the development of cancer and other genetic diseases. Some components of this network are classical DDR kinases such as ATM or ATR, which play a major role in orchestrating proteins involved in different pathways of the DDR. Many other kinases such as m-TOR and c-Abl, are not canonical components of the DDR, but have been shown to contribute to the fidelity of specific DDR pathways. We have generated a collection of 380 non-lethal kinase knockout cell lines by using the CRISPR/Cas9 technology in human cells. Our aim is to screen these different cell lines in a high-throughput manner using a panel of DNA-damaging chemotherapeutics, which target a variety of DDR pathways, and assess the viability of the cells. The data could allow us to 1.) discover novel roles of specific kinases, kinase families or pathways in the DDR and thus provide an interaction map for the scientific community 2.) discover novel drug-gene interactions which result in an increased
sensitivity or resistance to chemotherapeutics with a potential of finding use in diagnosis and treatment of cancer.

98 Wingless ligand Wnt5a promotes proliferation and inhibits apoptosis of first trimester trophoblasts

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Objective: Depending on the receptor context the wingless ligand Wnt5a affects non-canonical as well as canonical Wnt signaling thereby controlling different developmental processes. The presence of Wnt5a in decidual stromal cells suggested that the ligand could regulate early placental development and trophoblast behavior. Hence, we herein studied the influence of Wnt5a on trophoblast proliferation and motility. Methods: Immunofluorescence staining and western blot analyses were used to determine Wnt5a-producing cell types in early placental tissues and cell lines. Gene silencing of Wnt5a using siRNAs in SGHPL-5 cells and treatment of placental explant cultures and primary isolated cytotrophoblasts (CTB) with recombinant Wnt5a were performed. Wnt5a-dependent effects on proliferation, apoptosis and motility were studied. Furthermore, Wnt5a dependent activation of signaling pathways was analyzed. Results: Wnt5a was detectable in mesenchymal cells and leucocytes of the villous core and the decidua, as well as in decidual glands, CTBs and the trophoblastic cell line SGHPL-5. Silencing of Wnt5a in SGHPL-5 cells decreased proliferation and cyclin D1 expression which could be reverted by supplementation of recombinant Wnt5a. Additionally, increased BrdU incorporation into villous and cell column trophoblasts of placental explant cultures was detectable upon Wnt5a treatment. Furthermore, knock down cells showed increased apoptosis. Wnt5a addition increased, and gene silencing decreased the motility of SGHPL-5 cells. Treatment of SGHPL-5 cells, CTBs and placental explant cultures with recombinant Wnt5a provoked induction of the MAP Kinase pathway. Conclusions: Expression data and functional studies suggest a major role of the Wnt ligand Wnt5a in maintaining proliferation of CTBs and protection from apoptosis via non canonical Wnt signaling and the MAPK pathway.

99 The role of the transcription factor TRIM28 in keratinocyte differentiation and epidermal barrier formation

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Keratinocyte (KC) differentiation is a complex process that involves precise adjustment of gene expression, tightly regulated by the activation of specific transcription factors (TFs), including KLF4, p63, AP1 and NF-κB. Deregulation of TF-activity has been shown to be associated with the vast majority of skin diseases. The TF TRIM28 is known to induce histone methylation (H3K9me3) thereby leading to
repression of gene expression. However, its function in the epidermis is so far completely unknown. To identify TFs that may be involved in KC differentiation we performed gene chip analysis of non-differentiated and differentiated human KC and used complex bioinformatics tools to predict TFs involved in KC differentiation. Using in silico analysis, we showed that TRIM28 is highly active in KC, preferentially targeting genes of non-differentiated KC. To verify these data, we performed TRIM28 immunostaining of healthy human skin and indeed found strong nuclear expression primarily in the upper layers of the epidermis. To further investigate its function in KC differentiation we performed siRNA mediated gene silencing in KC and established organotypic skin models with these cells. Knock down of TRIM28 in our skin model led to an impaired development of the epidermis. In ongoing experiments we will further explore the role of TRIM28 in KC differentiation and the formation of the epidermal barrier in more detail. Our preliminary data suggest an important role for the newly identified TF TRIM28 in KC differentiation and epidermal development.

100 Proliferation of progeria cells is enhanced by Lamina-associated polypeptide (LAP) 2alpha through expression of extracellular matrix proteins

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Lamina-associated polypeptide (LAP) 2alpha localizes throughout the nucleoplasm and interacts with the fraction of lamins A/C that is not associated with the peripheral nuclear lamina. The LAP2alpha-lamin A/C complex negatively affects cell proliferation. Lamins A/C are encoded by LMNA gene, a single heterozygous mutation of which causes premature ageing disease called Hutchinson-Gilford Progeria Syndrome (HGPS). This mutation generates the lamin A/C variant, progerin, which acts in dominant-negative fashion and induces various cellular defects including highly lobulated nuclei with thickened lamina, alterations in chromatin organization, gene expression, DNA repair, cell cycle control and eventually leads to premature senescence. Our data show that expression of progerin in primary patient fibroblasts and hTERT-immortalized skin fibroblasts leads to loss of LAP2alpha and nucleoplasmic lamins A/C, impaired proliferation and down-regulation of extracellular matrix components. Surprisingly, contrary to wild-type cells, ectopic expression of LAP2alpha in cells expressing progerin restores proliferation and extracellular matrix expression but not the levels of nucleoplasmic lamins A/C. We conclude that, in addition to its cell-cycle inhibiting function with lamins A/C, LAP2alpha can also regulate extracellular matrix components independent of lamins A/C, which may help explain the proliferation-promoting function of LAP2alpha in cells expressing progerin.

101 Cell type specific isoforms of thrombospondin-1


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INTRODUCTION: Thrombospondin-1 (TSP-1) is engaged in the biological processes of angiogenesis and hemostasis. Analysis of plasma samples of cancer patients after surgery revealed a full-length 185 kDa and a smaller 160 kDa TSP-1 isoform. The latter prevailed post surgery indicating that processing of TSP-1 to 160 kDa might be triggered. Therefore, we aimed at characterizing the potential source of the 160 kDa TSP-1 isoform. METHODS: TSP-1 secreted by human endothelial cells (ECs), fibroblasts, smooth muscle cells (SMCs) in culture or by platelets isolated from whole blood, was detected by immunoblotting. Furthermore, TSP-1 isoforms were assessed in co-cultures of either ECs or platelets with isolated leukocytes. The contribution of the neutrophil proteases elastase and cathepsin G was tested through application of purified proteases. RESULTS: Platelets and ECs consistently released full-length TSP-1 into the supernatant, but both cell types co-cultured with neutrophils led to the appearance of a 160 kDa TSP-1 protein. Moreover, incubation of platelet or endothelial 185 kDa TSP-1 with neutrophil elastase or cathepsin G resulted in proteolytic processing to 160 kDa TSP-1 fragments. Fibroblasts and SMCs secreted a smaller 140 kDa isoform in addition to the full-length TSP-1 differing in its molecular weight from the 160 kDa TSP-1 generated by neutrophils and found in human plasma. CONCLUSIONS: Platelets and ECs, which are the main producers of TSP-1, consistently secrete the full-length 185 kDa protein whereas fibroblasts and SMCs also produce a smaller 140 kDa isoform of TSP-1. Of note, in co-culture of ECs or platelets with neutrophils, the proteases cathepsin G and elastase could be identified as the major players involved in processing the 185 kDa TSP-1 to 160 kDa fragments comparable to the TSP-1 isoform detectable in post-operative human plasma. The impact of this proteolytic processing on TSP-1 function in hemostasis is currently under investigation.

102 Role of an apical pore tyrosine residue of human P-glycoprotein in substrate gating

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P-glycoprotein is an ATP-binding cassette multidrug transporter that actively transports chemically diverse substrates across the lipid bilayer. X-ray crystal structure of a eukaryotic homolog of human ABCB1 from Cyanidioschizon merolae (Kodan et al. PNAS 2014) addresses the aspect of substrate gating and identifies a tyrosine residue in the apex of the central cavity (Y358) as being part of the extracellular gate. We mutated the analogous tyrosine residue in human ABCB1 to alanine and characterized the mutant in rhodamine 123 (rh123) transport experiments. The mutant was also combined with positively charged selector residues that allow two pseudosymmetric binding modes of rh123 to be addressed individually (Parveen et al. Mol Pharmacol 2011). The wild type transporter and mutants were characterized in steady state and zero-trans efflux protocol. In the absence of an active transporter, cells show high loading and no efflux. In cells expressing high levels of an active transporter, steady state levels of loading are low and efflux is high. Plotting steady-state levels of uptake on the abscissa versus first-order-rate-constants on the ordinate yields a hyperbolic curve with asymptotes conforming to x-
and y-axis. When combining the Y310A mutation with the selector mutations Q773R and Q132R, a remarkable deviation was observed. While Q773R mutant was located on the hyperbolic curve for active transport. This means that efflux is higher than expected from a coupled active transporter. Interpretation to this increase in flux-rate indicates passive (leak) transport in ABCB1. Same behavior is only observed, when substrate is allowed to bind in mode 1, but not, when binding occurs in mode 2. Therefore, residue Y310 is involved in rh123 gating when binding occurs in mode 1, but not when binding occurs in pseudosymmetric mode 2. Supported by the Austrian Science Fund within the scope of “Spezialforschungsbereich”• SFB35 (project part 3506 to HHS, 3509 to PC and 3524 to TS).

103 Structural characterization of intermediate steps during substrate translocation in the aspartate transporter -GltPh

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Glutamate is the major excitatory neurotransmitter. After its release into the synaptic cleft it is taken up by the excitatory amino acid transporters (EAAT). This process is essential to prevent accumulation of neurotoxic concentrations of glutamate in the extracellular space. Mammalian EAATs couple uptake of one glutamate to co-transport of three Na\textsuperscript{+}, one H\textsuperscript{+} and counter transport of one K\textsuperscript{+} ion. Structural studies on the mammalian EAATs have been greatly facilitated by the crystal structures of an archaeal aspartate transporter homologue (GltPh). In the present study we have combined in-silico and in-vitro methods to characterize intermediate steps during substrate translocation by GltPh across the membrane. Key steps involve binding of sodium to the second sodium binding site and disruption of a salt-bridge interaction between the transport and trimerisation domains. We used steered molecular dynamics to simulate the process of internalization of substrate in GltPh and we identified T308 to play an important role in binding of the second sodium. Site directed mutagenesis of T308 resulted in decreased Km for substrate and sodium as measured by uptake assays performed in reconstituted proteoliposomes. We have also extended our findings to the mammalian glutamate transporter (EAAT3). To measure global ensemble conformational change we have used Lanthanide Resonance Energy Transfer (LRET). The conformational ensembles observed agree well with the available crystal structures of GltPh.

104 Evidence for a role of NF-kB in the induction of endothelial senescence by stress

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Replicative senescence (SC) is a phenomenon describing the cease of cell division. A premature cellular SC can be induced by environmental stress which contributes, in vivo, to patho-physiological processes. Recent evidence supports the notion that development of both types of SC is mediated by NF-kB contributing to induce SC associated secretory phenotype (SASP). This study aims to assess that in endothelial cell (ECs) the induction of premature SC might be mediated by NF-kB and therefore be prevented by its modulation. We exposed HUVECs to H2O2, TNF, high glucose or serum starvation. We assessed their effects on cell proliferation (Ki67), cell cycle inhibitors (p16, p21), activity of SC associated beta-gal (SA β-gal), ICAM-1 expression, reactive oxygen species (ROS) production, nitric oxide (NO) bioavailability, cell migration and NF-kB translocation. For the inhibition of NF-kB pathway we used PHA-408 or Plumericin (PL). All four forms of stress, led to a substantial decrease in cell proliferation and to an increase in the number of cells with SA β-gal or cell cycle inhibitor (p16, p21) positive cells, consistent with successful induction of SC. SASP induction was evident by increased levels of ICAM-1 expression, ROS formation and reduction in NO bioavailability. TNF, H2O2 and serum starvation led to an increase in the number of cells with NF-kB translocation. TNF and glucose increased the migratory activity of ECs. Inhibition of the NF-kB pathway prevented the increase in the number of ECs, positive for SA β-gal or p21 and the increase in ICAM-1 expression, ROS production and cell migration. PL rescued NO bioavailability. PL also prevented the TNF induced loss in proliferation which PHA could not. Our data provide evidence for a role of NF-kB signaling to the stress-induced development of endothelial SC and the SASP. PL not only targets the NF-kB dependent development of the SASP, loss in NO bioavailability but also prevent TNF-induced decline in cell proliferation.

105 Endoreduplication and Polyploidy in human Trophoblasts


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Endoreduplication and resulting polyploidy are common and well described in plants as well as in maligned tumours but rarely seen in healthy mammalian cells and only a few examples are described in humans. Earlier studies revealed polyploidy in murine placental trophoblasts, a highly invasive cell-type that is indispensable for a successful pregnancy as it is responsible for proper nutrient and oxygen supply for the foetus. Errors in trophoblast function have been implicated in the pathogenesis of pregnancy diseases such as pre-eclampsia or intrauterine growth restriction. To better understand the situation in human placental development and function, we here analysed the chromosomal constitution of primary human trophoblasts using FACS and MACS analysis, laser-scanning microscopy and 3D modelling. Furthermore, we examined the cell cycle progression by investigating the pattern of Cyclin and Cyclin-dependent kinase inhibitor (CKI) expression in trophoblast development using immunofluorescence and western blot. Our data clearly indicates that human trophoblasts duplicate (tetraploid) and even quadruple (octoploid) their whole genome during their differentiation from villous cytotrophoblasts (vCTBs) to invasive, extravillous trophoblasts (EVTs). Furthermore, the Cyclin and CKI expression pattern resembles that of endoreduplicating cells. To learn more about this highly interesting phenomenon and
its potential influence on trophoblast function, siRNA mediated knock-down of factors important for endoreduplication such as Cyclin E and p57 with subsequent analysis of proliferation, apoptosis, gene expression and invasion will be performed. In summary, our cogent data reveals a multiplication of the whole genome during human trophoblast development.

106 Non-invasive tissue specific reporter system for monitoring of skeletal muscle cell differentiation

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Detection of skeletal muscle differentiation plays a key role for tissue engineering. In this study, we developed a reporter system to investigate the effectiveness of murine skeletal muscle differentiation. The use of a tissue specific promoter combined with a non-invasive bioluminescence assay allowed the evaluation of biological processes during myogenic differentiation in 2D and 3D culture systems as well as in bioreactors. To construct the muscle creatine kinase (MCK) specific reporter vector (pE3MCK-MetLuc ± CMVe), triple tandem of MCK enhancer was positioned to its truncated basal promoter (87bp) upstream of either a secreted luciferase or a fluorescent protein. In addition, up to three different myogenesis related miRNA seed sequences were ligated into 3UTR of DNA, in order to develop tracking strategy by reducing background signals in non-muscle cells. Cells were transfected with indicated constructs by using lipofection. The reporter gene signal in the supernatant was either detected using a luminometer after conversion of substrate into light units (secreted luciferase) or by fluorescence microscopy (fluorescence protein). The constructs exhibited strong luciferase and fluorescence expression in cells undergoing myogenic differentiation compared to non-muscle cells. Reconstruction of pE3MCK-Metluc vector by ligating of miRNA seed sequences resulted in low background signals in non-muscle cells. Tissue-specific promoters combined with E-boxes and miRNA seed sequences allow the amplification of tissue specific signals as well as kinetic monitoring of myogenic differentiation in a nonsample-destructive manner.

107 Proteome analysis of testis from infertile protein C inhibitor deficient mice reveals predominant changes in Serpin processing and Prostaglandin metabolism

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Serine protease inhibitors have therapeutic potential in a variety of pathogenic processes, ranging from thrombosis, innate immune response to liver cirrhosis. To investigate the physiological effects of Protein C inhibitor (PCI, SerpinA5) and to identify potential target proteins in the mouse proteome which might be influenced by PCI, the serpin-gene was inactivated in a mouse model with the resulting phenotype of male infertility. In the actual report 2D DIGE analysis were applied to find molecular mechanisms for PCI
in male reproduction. Comparing the testis of three PCI knock outs with three wild type mice demonstrated similar protein pattern in their testis unless a massive upregulation of prostaglandin reductase 1 (10-fold; P < 0.002) and complete shifts in the molecular weights of serpinA1C and serpinA3K. All these PCI dependent proteome changes were immunological verified. This current data suggest that the inactivation of serpinA5 strongly influences the isoform pattern of other A-clade serpins and the prostaglandin metabolism in testis.

108  Repurposing of drugs for pharmacological stimulation of hematopoietic progenitor cell transplantation

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Murine and human hematopoietic progenitor cells (HPCs) express Gs-coupled prostaglandin receptors. HPCs are amendable to in vitro stimulation by pharmacological means. We showed that if HPCs were in vitro incubated with treprostinil, a stable analogue of prostacyclin, their subsequent engraftment in lethally irradiated recipient mice was enhanced. The effect was further substantiated if recipient mice were in vivo treated with treprostinil. Stromal cell-derived factor 1 (SDF-1/CXCL12) and its cognate receptor CXCR4 are key factors to govern migration and homing of HPCs. Our data pointed that treprostinil upregulated CXCR4 expression in HPCs which was associated with enhanced in vitro migration towards SDF-1 as well as increased in vivo homing of these cells in to the bone marrow niche. CXCR4/SDF-1 interactions might be disturbed by the action of dipeptidyl peptidase (DPP)-4. This enzyme is expressed in HPCs and cleaves SDF-1. Accordingly, we found that vildagliptin, a well-known DPP4 inhibitor, enhanced therefore in vitro migration, in vivo homing and engraftment of HPCs. Surprisingly, combined application of treprostinil and vildagliptin rather reduced migration, homing and engraftment of HPCs as compared to the single administration of each drug. On the long run, our data might provide the basis for new therapeutic application of well-known drugs, here in particular to shorten the time period until the onset of the transplant of HPCs.

109  Identification of monogenic causes of chronic early-onset diarrhea using next generation sequencing


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Chronic diarrhea during infancy can be a manifestation of a variety of underlying conditions, including early-onset inflammatory bowel disease (EO-IBD) and non-inflammatory diseases such as congenital chloride or sodium diarrhea. Some of these conditions can be attributed to single gene defects as evidenced recently with the identification of human IL10-receptor deficiency. Our laboratory focuses on the identification of the monogenic causes for such disorders using high-throughput genomic approaches such as next generation sequencing (NGS). We here studied a patient from a consanguineous background presenting with an early-onset chronic non-mucoid and non-bloody diarrhea. Combined homozygosity mapping and exome sequencing enabled us to identify a novel nonsense mutation in the gene diacylglycerol-acyltransferase-1 (DGAT1). The mutation we identified segregated in an autosomal recessive manner, with both parents being heterozygous carriers and non-affected siblings being carriers or wild-type. The corresponding protein product diacylglycerol-acyltransferase-1 is involved in the terminal step of triglyceride synthesis. A deleterious biallelic splice-site mutations in DGAT1 has recently been identified to underlie a congenital diarrheal disorder in two siblings, which has been the only report on this disorder to date. We aim to further characterize how defective DGAT1 protein leads to the described phenotype, which is currently poorly understood. Our findings illustrate that chronic early-onset conditions affecting the homeostasis of the gut can be monogenic in nature, and exemplifies the need to correctly identify the molecular causes of early-onset diarrhea in order to enable appropriate and timely intervention for the severe clinical phenotypes accompanying chronic diarrhea.

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Enrichment strategy for malondialdehyde-modified proteins

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Oxidative stress is one of the major factors involved in the pathogenesis of inflammatory diseases and has been linked to many systemic pathologies including cardiovascular and neuro-degenerative diseases and cancer. Reactive oxygen species (ROS) lead to the oxidative breakdown of lipids and the subsequent formation of highly-reactive lipid peroxidation products. One of the most abundant such products is malondialdehyde (MDA). MDA forms covalent adducts on the primary amino group of exposed lysine residues present on the surface of proteins. These modifications act as danger-associated molecular pattern molecules (DAMPs) and are recognized by various mediators of the immune system, thereby eliciting a sterile inflammatory response. Yet, the subset of proteins and specific sites sensitive to modification by MDA are not well characterized.

Here, we present a two-step strategy for the enrichment of MDA-modified proteins and peptides for subsequent identification by mass spectrometry. In the first step, MDA-carrier proteins are enriched via
immunopurification using antibodies against MDA modifications. After tryptic digestion, MDA-modified peptides are enriched using hydrazine-functionalized agarose beads. Carboxyl-containing MDA modifications covalently couple to the beads forming hydrazones, and can be released by acid elution which allows immediate mass spectrometric analysis. The enrichment strategy allows the identification of MDA-carrier proteins and the exact determination of the site of modification. MDA-carrying peptides can be enriched up to 20-fold over the original peptide mixture in a spiked test sample. As a proof of concept, MDA-carrying proteins have been enriched from serum from healthy donors, however, the method can be applied to analyse a variety of samples, including clinical samples from chronically inflamed tissues, where MDA modifications are likely to play a role in eliciting and sustaining the inflammatory response.

111 Exome sequencing reveals a heterozygous RIT1 mutation in a fetal case of Noonan syndrome with gonadoblastoid testicular dysplasia

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Background: Exome sequencing is a novel and powerful tool to identify disease causing mutations in fetuses with malformations and unrevealing molecular karyotype. We applied exome sequencing in a fetus with hydrops, congenital heart disease and gonadoblastoid testicular dysplasia. Methods: Whole exome sequencing of a fetal sample was conducted on a HiSeq 2000. Confirmation analysis was performed by using conventional Sanger sequencing. Results: Whole exome sequencing revealed a pathogenic mutation in the RIT1 gene (c.270G>A (p.Met90Ile)), leading to the diagnosis of Noonan syndrome type 8. Comparison with the parents revealed that the mutation occurred de novo in the fetus. Conclusions: Here we present the first case of a fetus with RIT1 mutation leading to fetal hydrops and lethal outcome. Furthermore, the combination of heart malformation and fetal hydrops together with gonadoblastoid testicular dysplasia may represent a distinct subtype of Noonan syndrome. This case demonstrates the power of exome sequencing to identify disease causing mutations in unclear cases to establish the recurrence risk for further pregnancies.

112 Molecular characterization of Ccdc181, a Hook1 and microtubule interacting protein
Murine Hook1 is a known microtubule associated protein that is important for the morphological development of spermatozoa. This is supported by the abnormal-spermatozoon-head-shape (AZH) mutant, carrying a spontaneously mutated Hook1 gene which leads to malformed sperm heads and detached flagella. A previous experiment identified the so far uncharacterized protein Ccdc181 as a putative Hook1 interacting protein by a yeast-two-hybrid (Y2H) assay. Therefore, the aim of this project is the molecular characterization of Ccdc181 and the verification of its interaction with Hook1. As initial experiments northern blots and reverse transcription PCRs (rt-PCR) were performed to investigate the expression of Ccdc181 in different tissues as well as during testis development. These experiments revealed a so far unknown splice variant of Ccdc181. The putative interaction of Ccdc181 with Hook1 was verified and further narrowed down by Y2H, co-immunoprecipitation (Co-IP) and fluorescence resonance energy transfer (FRET) experiments. In contrast to what was previously hypothesized, experiments revealed that Ccdc181 interacts with the microtubule-binding-domain of Hook1 rather than with its organelle-binding-domain. Immunofluorescence (IF) experiments using NIH3T3 cells transiently overexpressing Ccdc181 showed a co-localization of Ccdc181 with the microtubular network. Moreover, this co-localization was also observed in developing spermatids in which Ccdc181 decorates the microtubular manchette, a structure important for the morphological development of spermatozoa. This putative interaction between Ccdc181 and microtubules was verified by a microtubule-binding assay. Ongoing experiments are designed to further elucidate the function of Ccdc181 by a CRISPR/Cas9 mediated knockdown in murine cell lines.

113 The molecular characterization of Hook1 and its putative interacting protein Ift81 in haploid male germ cell differentiation

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Murine Hook1 plays an essential role in spermatogenesis, because the truncation of Hook1 in the abnormal-spermatozoon-head shape (AZH) mouse mutant results in sperm lacking normal function and morphology. It has already been shown, that Hook1 binds microtubules and that it may play a role in
intra-manchette transport processes. However, the exact function of Hook1 is still unclear. Therefore, a Yeast-two-hybrid (Y2H) screen was performed to identify putative interacting proteins. By this approach Ift81 was found as a putative interaction partner of Hook1. Ift81 has been shown, to play an important role in the intraflagellar-transport (IFT), which is required for cillum formation. The aim of this project was to further characterize the function of Hook1 and Ift81 during spermatogenesis and to verify the putative interaction between them. For that purpose, the expression of Ift81 and Hook1 at RNA and protein levels was analyzed using testis of mice of different ages. Additionally, GFP- or dsRed-tagged fusion proteins of Ift81 and Hook1 were used to analyze their localization in cell culture. Furthermore, specific antibodies were used to analyze the localization of Ift81 and Hook1 in male germ cells. To verify the putative interaction of Hook1 with Ift81, Y2H assays, Co-immunoprecipitation and fluorescence resonance energy transfer (FRET) experiments were performed. Thereafter, the responsible interacting domains of Hook1 and Ift81 will be analyzed. To further study the biological function of Ift81 in cell culture a siRNA mediated knock-down in the murine cell line NIH3T3 will be performed.

114 Transcriptional regulation of SPAG6 by DNA methylation in NSCLCs

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Epigenetic abnormalities, especially DNA methylation, are involved in the pathogenesis of non-small cell lung cancers (NSCLC). By using a genome-wide approach, we recently identified ~ 500 tumor-specifically methylated genes in a large number of NSCLC patients. From most of them tumor-specific methylation in NSCLCs was unknown so far. One of these genes is the Sperm Associated Antigen 6 (SPAG6) gene. By analysing publically available IlluminaHiSeq RNA-seq data, we observed that SPAG6 mRNA expression is frequently lost in primary tumor (TU) compared to corresponding non-malignant lung tissue (NL) samples of NSCLC patients. In addition, NSCLC patients with squamous cell carcinoma subtype with low SPAG6 mRNA expression levels showed a shorter overall survival compared to NSCLC patients with high SPAG6 mRNA expression levels. We observed loss of SPAG6 expression in 5 NSCLC cell lines compared to
normal human bronchial epithelial cells (NHBEC). Subsequently, we treated NSCLC cells with the epigenetically active drugs 5-aza-2-deoxycytidine and Trichostatin A and observed SPAG6 reexpression. Bisulfite genomic sequencing of parts of the 5’ region of SPAG6 revealed that the vast majority of CpG sites are methylated in NSCLC cells which do not express SPAG6 while no methylation was found in NHBECs. Moreover, we analysed SPAG6 methylation in TU and corresponding NL samples of 147 stage I-III NSCLC patients using methylation-sensitive high-resolution melting (MS-HRM) assays. Differences in SPAG6 methylation between TU and corresponding NL samples were statistically significant (p < 0.0001) demonstrating that SPAG6 is tumor-specifically methylated. We additionally investigated SPAG6 protein expression in TU and NL samples of 35 NSCLC patients by immunohistochemistry. In the vast majority of TU samples SPAG6 protein expression was lost in tumor cells but was observed in bronchial and bronchiolar epithelial cells of NL samples. Overall, our results demonstrate that DNA methylation is the major mechanism for frequent loss of SPAG6 expression in NSCLCs.
Poster Session “Molecular Pharmacology”, 15.00 - 17.00
Chaired by Michael Freissmuth and Stefan Böhm

115 Which SEC24 isoform is required for ER Export of G protein-coupled receptors?
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116 'Second-generation' mephedrone analogs, 4-MEC and 4-MePPP, differentially affect monoamine transporter function

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The nonmedical use of synthetic cathinones is increasing on a global scale. 4-Methyl-N-methylcathinone (mephedrone) is a popular synthetic cathinone that is now illegal in the United States and other countries. Since the legislative ban on mephedrone, a number of 'second-generation' analogs have appeared in the street drug marketplace, including 4-methyl-N-ethylcathinone (4-MEC) and 4'-methyl-α-pyrrolidinopropiophenone (4-MePPP). Here we characterized the interactions of 4-MEC and 4-MePPP with transporters for 5-HT (SERT) and dopamine (DAT) using molecular, cellular, and whole-animal methods. In vitro transporter assays revealed that 4-MEC displays unusual 'hybrid' activity as a SERT substrate (ie, 5-HT releaser) and DAT blocker, whereas 4-MePPP is a blocker at both transporters but more potent at DAT. In vivo microdialysis experiments in rat brain demonstrated that 4-MEC (1-3mg/kg, i.v.) produced large increases in extracellular 5-HT, small increases in dopamine, and minimal motor stimulation. In contrast, 4-MePPP (1-3mg/kg, i.v.) produced selective increases in dopamine and robust motor stimulation. Consistent with its activity as a SERT substrate, 4-MEC evoked inward current in SERT-expressing Xenopus oocytes, whereas 4-MePPP was inactive in this regard. To examine drug-transporter interactions at the molecular level, we modeled the fit of 4-MEC and 4-MePPP into the binding pockets.
for DAT and SERT. Subtle distinctions in ligand-transporter binding were found that account for the differential effects of 4-MEC and 4-MePPP at SERT. Collectively, our results provide key information about the pharmacology of newly emerging mephedrone analogs, and give clues to structural requirements that govern drug selectivity at DAT vs. SERT.

117  **Zinc slows down partial reactions in the transport cycle of DAT**  
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118  **The conformational changes of the human dopamine transporter during the transport cycle**  
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Dopamine is a neurotransmitter involved in addiction, learning and memory, it is translocated by the human dopamine transporter [hDAT] through coupling to the transmembrane sodium gradient. Zinc is thought to regulate dopamine, as it has been found to inhibit hDAT at physiological concentrations (10-30 µM). Here we begin to determine the effect of mutations and zinc on the conformation of hDAT. The mutations are clustered into two groups; the intracellular hydrophobic lid (Y335A and T432F) and the second ion lock (D59A, E61A and R443A). The mutations that disrupt the intracellular hydrophobic lid have been previously found to drastically reduce uptake activity of substrate by hDAT. hDAT activity can be rescued to near wild-type levels through the addition of zinc, in a dose-dependent manner. Our data show that those mutations in the second ion lock have been found to have similar (to wild-type) substrate uptake by hDAT, in the absence or presence of zinc, but the triple mutant (D59A_E61A_R443A) has been found to reduce amphetamine-mediated efflux in the presence of zinc to near-basal levels. In order to further investigate the conformational effects of mutations, substrates and inhibitors on the conformational cycle, the development of mutations that allow for facile labelling for use in spectroscopic investigations are to be selected. The primary spectroscopic data (from EPR and FRET) will be used in combination with molecular dynamics simulations to elucidate structural changes during the hDAT transport cycle.

119  **Profound releasing activity of mephedrone metabolites explains the sustained mephedrone action**  
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Introduction: 4-Methyl-N-methylcathinone (mephedrone, [MEPH]) is a popular psychostimulant and one of the major representatives of the former legal highs which were sold as “bath salt” or “plant food” over the internet. MEPH impinges on monoaminergic signaling in the brain in an amphetamine-like fashion and most closely resembles 3,4-methylenedioxymethamphetamine (MDMA, “ecstasy”). Thus, MEPH acts as a competitive inhibitor at the high-affinity monoamine transporters for dopamine ([DA], dopamine transporter, [DAT]), serotonin ([5HT], serotonin transporter, [SERT]) and norepinephrine ([NE], norepinephrine transporter, [NET]) and induces efflux of DA, NE and 5HT into the synaptic cleft via their cognate transporters. However, given the discrepancy between the long duration of the MEPH-induced “high” and the plasma half-life, we tested whether MEPH exhibits psychoactive metabolites which account for the long psychoactive action of this drug. Methods: Nor-MEPH, hydroxytolyl-MEPH and dihydro-MEPH were synthesized in racemic mixtures and tested for their activities on DAT, NET and SERT in vitro, ex vivo and in vivo. Results: Nor-, hydroxytolyl- and dihydro-MEPH were found to inhibit uptake mediated by DAT, NET and SERT with IC50 values in the low micromolar range. Moreover, Nor- and hydroxytolyl-MEPH were capable of inducing efflux via all three monoamine transporters. Consistent with in vitro findings, the latter mentioned metabolites mimicked the action of the parent compound in vivo. Conclusion: The fact that MEPH is subject to a fast metabolic turnover, yet induces a long lasting high appeared to be inconsistent. However, our data indicate that the presence of MEPH per se is not the only parameter determining the stimulating effect of this drug. As it has been shown for MDMA, MEPH exhibits metabolites which elevate the synaptic concentrations of DA, NE and 5HT and thus might account for the prolonged psychostimulant properties of MEPH.

120 Enhancement of colchicine fluorescence by metal ions

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Colchicine, is a toxic natural product and secondary metabolite, initially extracted from plants of the genus Colchicum. It was utilized originally to treat rheumatic and inflammatory complaints. It was also prescribed for its cathartic and emetic effects, and used in the treatment of acute gouty arthritis and familial mediterranean fever. It has also medicinal applications in the treatment of some auto-immune and dermatologic disorders. It has diverse effects on tubulin such as microtubules assembling [1-4]. Colchicine, like many other cytotoxic drugs, enters the cell through the lipid bilayer by passive diffusion and binds reversibly to P-glycoprotein (Pgp) [5]. At a molecular level, colchicine inherently has too low intrinsic fluorescence intensity and the fluorescent effect of colchicine binding with tubulin is used for tracing of components in the cells. Due to the importance of using Colchicine in pharmacology and its widespread application, to enhance its intrinsic fluorescence for better detection of this compound when
it attaches to tubulin proteins, its complexation with some common metal ions (Li, Na, K, Mg, Ca, Sr, Cr,
Mn, Fe, Ni, Co, Cu, Ag, Cd, Hg, Al, Pb) was studied by fluorescence spectroscopy. Because the cations
offer an effectual means of probing the role of metal cation size in the structural organization of
biological membranes. The molar composition of the complex was determined according to the mole-
ratio method which corresponding to the formation of a 1:1 complex between Colchicine and silver ions.
The experimental results revealed silver cation has an ability to increase the intrinsic fluorescence of
Colchicine to quintuple.

121 Comparison of antioxidant enzyme activities between Colchicum crocifolium and Colchicum
kotschyi

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Comparison of antioxidant enzyme activities between Colchicum crocifolium and Colchicum
kotschyi

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Colchicum L., is a very interesting monocotyledonous geophyte plant because of its unusual life cycle. The
autumnal one followed by the winter season and photosynthetically active one which leads ageing and
dormancy. Different researches described Colchicum L., as a source of therapeutically active
alkaloids called colchicinoids. One of the most plentiful alkaloid- colchicine, is known to have
cancerostatic, anti rheumatic, anti inflammatory, anti mitotic, cathartic and emetic effects. The main
substrates for colchicines’s biosynthesis are dopamine and cinnamic acid. The polyphenol oxidase is
expected to participate in dopamine formation from tyrosine. The authors were interested in the
comparative study on some antioxidant enzyme activities in different organs of spring- flowering species
(C. crocifolium Boiss) and autumn- flowering species (C. kotschyi Boiss). The results showed that the
highest and the lowest polyphenol oxidase activities were noted in the roots and seed of C. kotschyi
correspondingly. The peroxidase activity was maximum at the roots of C. crocifolium, however the
lowest peroxidase activities were observed in the daughter corm in C. crocifolium. The Superoxid
dismutase activity in all of the organs in C. crocifolium and C. kotschyi was identical and on the other
hand it was negligible in the seed

122 Determination of protein contents in different organs of Colchicum varians

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Colchicum L., that has an uncommon life cycle, is a monocot geophyte. Its life cycle containing of two
growing phases: autumnal period followed by winter season and photosynthetically active period being
expected to result in senescence and latency. After the starch, protein content characterized as the
second vital storage compound in corms. Soluble and insoluble protein contents of the seed, roots,
mother and daughter corms, leaf and stem of spring flowering species of genus Colchicum (C. varians Freyn & Bornm) in both quantity and quality aspects were studied. Results showed that the seed had the largest soluble protein content since roots had the least amount of soluble protein. Interestingly, the authors determined a lower level of insoluble proteins than soluble proteins in all examined organs except stem and leaf. These results confirm that the function of total protein in any organs depends on the stages of the plant lives. Because of the active phase of plant life and flowering and photosynthesis is ongoing, the amount of total protein in the mother corm and roots is lower than the other organs.

123 Studies of Colchicine content and some antioxidant enzyme activities in Colchicum varians

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Colchicum L., is a monocot geophyte with an uncommon life cycle. Its life cycle containing of two growing phases: autumnal and photosynthetically active period. The medicinal properties of some species of Colchicum L., have been known over than 3000 years. The medicinal value of the genus Colchicum is recognized to the presence of colchicine with low intrinsic fluorescence intensity. Fluorescence spectroscopy, is an influential tool for determination of the reactivity of chemical and biological systems since it allocate non-intrusive measurements of substances in low concentration under physiological states. The key substrates for colchicine’s biosynthesis are dopamine and cinnamic acid. The polyphenol oxidase is predictable to participate in dopamine formation from tyrosine. The authors were interested in the comparative study on Colchicine concentrations of the seed, corms and vegetative organs of spring- flowering species of Colchicum (C. varians Freyn & Bornm) by means of fluorescence spectroscopy. Although polyphenol oxidase, Peroxidase and Superoxid dismutase activities in aforementioned organs were determined. The results showed that, the highest and the lowest amount of colchicine were observed in the seed and roots respectively. The results of qualitative and quantitative analysis of antioxidant enzyme activities showed that the highest and the lowest polyphenol oxidase activities were noted in the mother corm and the roots correspondingly. The peroxidase activity was maximum at the mother corm, however the lowest peroxidase activities was observed in the stem. The Superoxid dismutase activity in all of the organs were close to each other, however, the leaves showed higher superoxid dismutase activity.

124 Evaluation of Colchicine contents between Colchicum crocifolium and Colchicum kotschyi by Fluorescence spectroscopy

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Colchicum L., is an absorbing monocot geophyte plant because of its exceptional life cycle. The autumnal one followed by the winter season and photosynthetically active one which conducts ageing and stagnation. Several organs of Colchicum L., especially corms and seeds were used in medicine for more than 3000 years. The medicinal value of the genus Colchicum is imputed to the presence of colchicine with low intrinsic fluorescence intensity. Fluorescence spectroscopy is an influential tool for the study of the reactivity of chemical and biological systems since it dedicate non-intrusive measurements of substances in low concentration under physiological states. The standard addition method is used as a technique of the standard preparation for overcoming complicated environmental impacts on fluorescence analytical signals. Colchicine concentrations of the seeds, corms and vegetative organs of one spring-flowering species of Colchicum (C. crocifolium Boiss) and one autumn-flowering species of Colchicum (C. kotschyi Boiss) were analysed by means of fluorescence spectroscopy. Colchicine was extracted from fresh organs using standard methods described by Koehler and Franz. The results showed that, the highest and the lowest amount of colchicine were observed in seed and mother corm of C. crocifolium respectively. The standard addition method allows making measurements of standards in a solution having the same matrix composition as the unknown sample. According to the obtained results, the amount of colchicine in the seeds was higher than the other organs which confirm the previous studies. Further reviews on the other species could help to figure out the highest colchicine content in the other organs of different species to compare with Colchicum autumnale as a source of colchicine.

125 Identification and evaluation of pharmacological chaperones with target specific action on the bile salt export pump (BSEP/ABCB11)


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The bile salt export pump (BSEP/ABCB11), a member of human ABC family, is localized at the apical membrane of hepatocytes and transports bile salts into canaliculi. A number of non-synonymous mutations have been indentified which interfere with correct folding and thereby lead to progressive familial intrahepatic cholestasis type II (PFIC II). The E297G and D482G are the most frequent mutations found in the European population, jointly contributing to approximately 60% of all PFIC II cases. Hence, this study aimed at identifying small molecule correctors (pharmacological chaperones) that specifically bind to and rescue mutant misfolded protein to the plasma membrane, thereby curtailing or eliminating the disease phenotype. We have generated mutations E297G, E482G, R517H and R1153C in human BSEP. HEK293 cells were transfected and stable cell lines expressing BSEP mutants were generated.
These non-synonymous mutations can be rescued by temperature shift to 28°C. Cyclosporine A (CsA) and pioglitazone, which are known to cause cholestatic liver injury, were used as controls. While pioglitazone was shown to be capable of correcting folding and rescuing mutant E297G in a dose dependent manner. Cyclosporine A trapped the mutant in the endoplasmic reticulum. The latter data are in agreement with an earlier report by Román et al (Tox Sci 2003). A pharmacophore model was derived from a data set of compounds known to cause intrahepatic cholestasis, using a machine learning protocol. This model was used to screen the drug data bank. The top 10 hits from an in silico screen were tested for their BSEP inhibitory (using [3H]Taurocholate (TCA) uptake in inverted membrane vesicles) as well as their pharmacochaperone activities. Results indicate that bromocryptin inhibits TCA uptake and also increases the surface expression of the ABCB11-E297G mutant. This provide a proof of principle that pharmacochaperones can be found among compounds with BSEP inhibitory activity.

126 Transmembrane proteins of Fasciola hepatica: identification and characterization of new putative drug targets


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Fasciola hepatica, a parasitic flatworm (phylum Platyhelminthes, class trematode, subclass Digenea, family Fasciolidae), is the cause of one of the most important diseases affecting animal health all over the world, causing the so-called liver fluke disease (fascioliasis ). Triclabendazole (TCBZ) has been the drug of choice for the treatment of fascioliasis for more than 25 years because of its high activity against both adult and juvenile flukes. However, during the last decades, there are an increasing number of reports on drug resistance against TCBZ in Fasciola hepatica. Possible molecular mechanisms underlying TCBZ resistance could be mediated by one of the following mechanisms: (i) accelerated metabolism of drugs, (ii) mutations in target proteins which eliminate drug sensitivity, (iii) accelerated drug efflux via transmembrane transporters of the ABC family. The last option is common in nature and was therefore suspected to play a significant role in drug resistance against TCBZ in F. hepatica. We performed next-generation sequencing (NGS) to identify new ABC transporters of F.hep and mutations in these transporters that could confer resistance to TCBZ. For this approach TCBZ-resistant and susceptible adult flukes from Northern Ireland and Lower Austria were used. In parallel, we also generated antibodies against putative ABC transporters of F. hepatica. Additionally, cells were transfected with ABC transporters to perform cell viability assays (CVA). The results from both bioinformatics part and functional analysis will probably shed light on TCBZ drug resistance in F. hepatica.

127 Target Deconvolution of Immunosuppressive Circular Plant Peptides

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Cyclotides are head-to-tail cyclized cysteine-rich plant peptides of up to 35 amino acids in size, which contain a characteristic cyclic-cystine-knot motif. Their stabilized framework confers cyclotides a promising template for peptide epitope grafting in drug development [1]. We have recently shown that the cyclotide kalata B1 exhibits promising anti-proliferative, but non-cytotoxic effects on CD3/CD28 activated human T-lymphocytes and therefore this peptide has been further explored for its immunosuppressive activity [2]. An active lysine mutant [T20K]kalata B1 was selected and chemical functionalization with an affinity label enabled peptide immobilization on a solid support. The peptide probe was utilized for affinity chromatography using T-lymphocyte extracts to pull-down and to identify its molecular target. In addition target deconvolution was assisted by the use of cyclotides comprising a photo-crosslinker as well as an affinity label for photo-affinity pull downs. All modified cyclotides were characterized in vitro for their cytotoxicity, stability against proteolytic enzymes and anti-proliferative activity for validation of their therapeutic potential. In summary we are developing a target deconvolution strategy for immunosuppressive cyclotides using a combined peptide chemistry and chemical proteomics approach. This may essentially be useful to elucidate the mode-of-action of cyclotides, but may also have broader applications to assist target discovery of other therapeutic agents.

Acknowledgements: We would like to thank Gernot Schabbauer (Medical University of Vienna) for help with anti-proliferative assays and Christian Becker (University of Vienna) for help with peptide synthesis. This work has been supported by the Austrian Science Fund – FWF (P24743-B21) and by the Austria Wirtschaftsservice GmbH (P1308423). References:n[1] Craik, J.D., (2013), J. Pept. Sci., 19, 393-407. [2] Gruendemann, C. et al., (2013), PLoS One, 8, e68016.

128 Fast and Slow Repriming of Voltage-Gated Na+ Channels by Lidocaine

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The clinically important suppression of high-frequency discharges of excitable cells by local anesthetics (LA) is largely determined by drug-induced prolongation of the time course of recovery from inactivation. Here, we systematically investigated the effect of the LA lidocaine on recovery from inactivation produced by short (50 ms, R-50) and by long (10 s, R-10) inactivating prepulses in heterologously expressed rNaV1.4 wild-type channels and in mutations of the domain IV S6 segment, an important determinant of drug-binding and gating. Following R-50 wild-type channels rapidly recovered from one inactivated state (IF), whereas with R-P10 recovery occurred mainly from two slower inactivated states (IM, IS). With R-50 lidocaine (500 µM) increased the time constant of recovery (I-REC) from IF and introduced a slow phase of recovery with kinetics similar to IM (IM-LIDO). With R-10 lidocaine increased I-REC IM (IM-LIDO). Most of the investigated mutations altered the kinetics of IF and IM. The mutation-
specific changes in I-REC IM-LIDO and I-REC IM-LIDO were not correlated with each other, suggesting that, despite similar kinetics, both processes occur by different mechanisms. However, we found a significant correlation between I-REC IM and I-REC IM-LIDO confirming previous reports that lidocaine binds with high affinity to [I(M)]. We also present evidence that the drug-induced increase in I-REC IF results from binding to a low-amplitude slow inactivating state during R-50. This state may be a novel drug target for suppression of high-frequency discharges.

129 The kinetic basis of selective ligand recognition in the monoamine transporters


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The plasmalemmal transporters for the monoamines serotonin (SERT) and dopamine (DAT) are critical for homeostatic control of neurotransmission. Their role in the modulation of a plethora of brain functions is reflected by the psychoactive effects of their competitive inhibitors (e.g. tricyclic antidepressants, methylphenidate, and cocaine) and exogenous substrates such as amphetamines. Upon rapid application of substrate, SERT- and DAT-expressing cells display an inwardly directed current comprised of a peak- and a steady-state component. These currents can be utilized to decipher the kinetic basis of selective inhibitor binding and substrate transport with high temporal resolution and without the need of high-affinity radioligands as surrogate. Our analysis of inhibitor binding shows that the selectivity of methylphenidate and desipramine for DAT and SERT, respectively, can be accounted for by their rate of association, and not by the residence time in their respective binding sites. Furthermore, we resolved the kinetics of extracellular substrate binding and translocation and provide estimates for intracellular substrate-binding rates. These data will help to unravel the molecular underpinnings of ligand selectivity and the actions of exogenous substrates, such as amphetamines, at the monoamine transporters.

130 Mechanism of Low-Efficacy Substrate Efflux at the Human Serotonin Transporter

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The serotonin transporter (SERT) terminates serotonergic synaptic transmission by reuptake of serotonin from the synaptic cleft. SERT is, among other transporters, target of a plethora of psychoactive substances. For example, exogenous substrates like methylene-dioxymethamphetamine (ecstasy) increases extracellular serotonin concentrations via SERT-mediated serotonin efflux. Recently, a class of compounds was identified, which exhibit low efficacy in inducing neurotransmitter efflux as compared to
full substrates. These substances were thus termed partial releasers. Given their low efficacy and mild psychoactive actions, they have been discussed as potential therapeutic agents for the therapy of substance abuse. However, it has not yet been possible to explain the mechanism of this partial efficacy. The transport cycle of SERT can be studied by electrophysiological means. Using this technique, we analyzed PAL-1045 as an example of a partial releaser for SERT. Results from our experiments suggest that PAL-1045 readily diffuses through the membrane and displays high affinity for the inward facing conformation of SERT. These attributes translate into a longer dwell-time in its binding site, which precludes intracellular serotonin binding and thus efflux. Taken together, we provide evidence for a mechanism of low-efficacy efflux in SERT. These results have implications for the development of low-efficacy releasers as therapeutic agents for addiction therapy.

131 Exocyst-dependent trafficking of the wild type dopamine transporter (DAT) and folding-deficient DAT mutants


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Poster Session “Public Health”, 15.00 - 17.00
Chairied by Martin Posch

132 The influence of mortality time-points on pooled effect estimates in critical care meta-analyses

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BACKGROUND: There is an on-going debate among meta-analysis methodologists and statisticians whether it is appropriate to pool mortality estimates from clinical trials that used mortality outcomes ascertained at different time-points. If the relative effects vary over time, which might especially be the case in critical care, standard pooling of studies with different follow-up times within one meta-analysis would not be justifiable. OBJECTIVES: Describe the current practice of dealing with different mortality time-points and analyze the influence of different time points on pooled effect estimates in actual Cochrane critical care meta-analyses. METHODS: The Cochrane Database of Systematic Reviews was searched for critical care-reviews. Meta-analyses were recalculated using all described strategies and influence of such strategies on deviation of pooled effect estimates compared to a "use last time-point available"-approach was analyzed using meta-regression and multilevel mixed-effects linear regression.
RESULTS: 835 reviews were evaluated, 80 meta-analyses of 298 studies, representing 107,605 patients were included. 49 (61%) reviews did not state any strategy, 9 (11%) used separate analyses for each time-point, 9 (11%) used the last available, 6 (8%) used a closest to defined time-point, 3 (4%) performed separate analyses for last and predefined, 2 (3%) mixed some, 1 (1%) computed pre-defined time-points from study-data, and 1 (1%) pooled all but performed a sensitivity analysis. Among 388 recalculated meta-analyses no influence of the strategies "pool short-, middle-, long-term", "use closest to defined" and "separate" on effect estimates was found compared to "use last available". COCNCLUSIONS: Reviews use a large variety of strategies to deal with different mortality time-points, however more than 50% don't even report any strategy for this problem. In summary, we found no influence of different strategies on effect estimates in critical care Cochrane reviews.

133 Perceived relevance of educative information on Public (Skin) Health in Austria: Aspects of preventive Gender medicine

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Unprotected leisure time exposure to ultraviolet radiation from the sun or artificial tanning beds is the most important environmental risk factor for melanoma, a malignant skin cancer with increasing incidences over the past decades. The aim of the present study was to assess the impact of skin health information provided by several sources and different publishing issues on knowledge, risk perception, and sun protective behavior of sunbathers. A cross-sectional questionnaire survey was conducted among Austrian residents (n=563) spending leisure time outdoors. Print media, television, and family were perceived as the most relevant sources of information on skin health, whereas the source physician was only ranked as fourth important source. Compared to other sources, information provided by doctors positively influenced participants' knowledge on skin risk and sun protective behavior resulting in higher scores in the knowledge test (p=0.009), higher risk perception (p<0.001), and more sun protection (p<0.001). Regarding gender differences, internet was more often used by males as health information source, whereas females were more familiar with printed information material in general. The results of this survey put emphasis on the demand for information provided by medical professionals in order to attain effective, long-lasting promotion of photoprotective habits.

134 Group sequential designs for Clinical Trials with multiple treatment arms

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For the comparison of several experimental treatments, doses or dose regimens to a control, recently several multi-armed group sequential clinical trial designs have been proposed. They allow one to stop randomization to individual arms early if the corresponding null hypothesis can be rejected in an interim
analysis. To minimize the required number of patients, we consider a variant of such designs where the overall trial stops as soon as for any of the arms the null hypothesis of no treatment effect can be rejected. While standard multi-armed group sequential designs control the type I error rate if such a stopping rule is used, they are typically strictly conservative. This can be explained by the fact that treatment arms for which no rejection can be achieved at the interim analysis could be further tested in the final analysis if the trial was continued. For the comparison of two experimental treatments to a common control we derive improved stopping boundaries that exhaust the type I error rate for stopping rules where the trial is stopped as soon as a null hypothesis can be rejected. Besides we search for optimized boundaries that minimize the expected sample size while maximizing the probability to show efficacy in both arms. We compare the operating characteristics of these optimized designs to standard multi-armed group sequential designs. Furthermore, several extensions as trials with futility boundaries and group sequential t-tests for small sample sizes are discussed. (FP7 project "Advances in Small Trials dESign for Regulatory Innovation and excellence" (ASTERIX) of the European Commission, Grant agreement no: 603160)

135 Evaluation of non-response bias in epidemiology: Example from the Mental Health in Austrian Teenagers (MHAT) Study

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Epidemiological research aims at providing precise prevalence estimates of a disease for the general population. Non-response is a known problem in epidemiology and might lead to an under- or overestimation of effects. The Mental Health in Austrian Teenagers Study is the first large epidemiological study on mental health problems and psychiatric disorders in adolescents aged 10-18 years in Austria. For the first screening stage, a total of N = 3610 adolescents were successfully recruited from schools whereas approx. 50% denied participation. About N > 400 participated in the subsequent interview stage, which was again about 50% of the planned sample size. Several methods for evaluating a possible non-response bias were applied including comparing response rates between subgroups, using data obtained from other informants (teachers) and comparing drop-outs and completers with respect to prior obtained data. Results revealed higher non-response for specific school types and grades. According to teachers’ records, non-respondents tend to show less effort and ability to concentrate in school and tend to be absent more often. However, effect sizes were quite low. Looking at the interview stage, there were no significant differences between those who participated and those who refused regarding mental health data obtained in the screening stage. Besides the use quantitative data, qualitative information (e.g. context of data collection, reasons for non-response) can also contribute to the evaluation of a non-response bias and foster learning for further studies. Doubts about data protection and anonymity and mental health as a topic itself turned out as one of the most frequent
reasons for refusal. To conclude, whereas there was evidence for a small non-response bias concerning the initial recruitment of participants leading to a possible underestimation of the prevalence estimates, no selective drop-out was observed from the first to the second stage of the study.

136 Antenatal infection screen-and-treat program in routine pregnancy care: evaluation of long-term outcomes

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Background: Vaginal infections in early pregnancy are associated with preterm birth. The long-term results of an antenatal screen-and-treat program for asymptomatic vaginal infections were evaluated.

Methods: Data of all women with singleton pregnancies who delivered at our tertiary referral center between January 2005-2014 were retrospectively evaluated. Women who presented for a planned birth at our department between 10+0 and 16+0 gestational weeks were assigned to the intervention group. They were routinely screened for asymptomatic infections using Gram stain and treated in case of bacterial vaginosis, candidiasis or trichomoniasis, according to our clinical protocol. Women who did not undergo the antenatal program served as controls. Pregnancy care was equal in the groups. Spontaneous preterm birth served as the primary outcome variable. Results: A total of 8490 out of 20052 (42.3%) women with singleton pregnancies underwent the program. The mean gestational week at delivery was 38.8±2.6 and 37.5±4.3 in the intervention and control group, respectively (p<0.001). The incidence of preterm birth was significantly lower in the intervention group, compared to the control group (9.7% vs. 22.3%; p<0.001). Moreover, stillbirth and late miscarriage occurred less frequently in the intervention group (p<0.001). Conclusions: Our long-term results support the use of an antenatal infection screen-and-treat program in routine pregnancy care, in order to prevent preterm delivery. This simple public health intervention could lead to a reduction of preterm delivery and improve pregnancy outcomes.

137 Barriers to research utilization among registered nurses from traditional Chinese medicine hospitals: a cross-sectional survey in China

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Background: Due to the increasing internal and external expectations of higher quality nursing, it’s no longer acceptable for nurses to deliver nursing care only on experience and textbook knowledge. Clinical nurses are expected to systematically gather best research evidence when they are making professional decisions. Despite the imperative for research utilization, many nurses, do not apply research findings to clinical practice. There were several investigations identifying perceived barriers to research utilization of nurses who working in general hospitals in China. However, there is no investigation focused on nurses working in traditional Chinese medicine hospitals. Compared with general hospitals in China, the ratio of doctors to nurses of traditional Chinese Medicine hospitals is low and the proportion of undergraduate registered nurses is also low. What’s more, most of them obtained their degree from non fulltime education. Hence, there might be some differences of relevance with regard to barriers of RU between RNs from the two kinds of hospitals. Methods: A multiple institutional cross-sectional survey design and a convenience sampling method were adopted. A total of 648 registered nurses from 4 tertiary level hospitals in capital city, Beijing, China, were recruited in a period from August 2014 to October 2014. A modified BARRIERS Scale and self-designed basic information questionnaire were used. Data were analyzed with descriptive statistics, t tests and one-way ANOVAs, and Spearman correlation analysis.

Results: Barriers belong to the research subscale was the top one. The lack of time on job was ranked as the top greatest barrier (58.8%), followed by the lack of knowledgeable colleague (57.5%) and overwhelming researches (53.7%). Clinical experience, working pressure, job satisfaction and research experience might be the factors associating perceptions of the barriers. Conclusions: RNs in traditional Chinese medicine hospitals felt higher barriers than other developed western countries and HongKong, China. Reducing RNs’ working pressure, promoting their positive attitude to nursing, enhancing research training might be helpful for research utilization. And further suggestion for up are building research utilization group with RNs with different working experience, cooperating with school of nursing closely and strengthening the research course during undergraduate education. This work is funded by the Young Talents Plan of Beijing Municipal Education Commission (2013-YETP-0796).

138 A decision theoretic approach to subgroup selection

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An important objective in the development of targeted therapies is to identify the populations where the treatment under consideration has positive benefit risk balance. We consider the setting, where the efficacy of a treatment is tested in an overall population as well as a pre-specified subpopulation. Based on a decision theoretic framework we derive optimized trial designs by maximizing utility functions. Features to be optimized include the sample size and the population the trial is performed in (the full
population or the targeted subgroup only). The approach accounts for prior knowledge on the efficacy of the drug in the considered populations using a two dimensional prior distribution. The considered utility functions account for the costs of the clinical trial as well as the expected benefit when demonstrating efficacy in the different subpopulations. Examples of optimized trial designs are determined by numerical optimization for several scenarios.

139  **Adapted Levels of Evidence for Small Populations**

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A full independent drug development programme to demonstrate efficacy may not be ethical and/or feasible in small populations such as paediatrics populations or orphan indications. Different levels of extrapolation from a larger population to smaller target populations are widely used for supporting decisions in this situation. There are guidance documents in drug regulation, where a weakening of the statistical rigour for trials in the target population is mentioned to be an option for dealing with this problem. To this end we propose clinical trials designs, which make use of prior knowledge on efficacy for inference. We formulate a framework based on prior beliefs in order to investigate when the significance level for the test of the primary endpoint in confirmatory trials can be relaxed (and thus the sample size can be reduced) in the target population while controlling a certain posterior belief in effectiveness after rejection of the null hypothesis in the corresponding confirmatory statistical test. We show that point-priors may be used in the argumentation since under certain constraints they have favourable limiting properties among other types of priors. The crucial quantity to be elicited is the prior belief in the possibility of extrapolation from a larger population to the target population. We try to illustrate an existing decision tree for extrapolation to paediatric populations within our framework.

140  **Adaptive designs for confirmatory model based decisions using MCP-Mod**

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Adaptive seamless designs for confirmatory clinical trials have attracted a lot of attention because they offer the possibility to combine different phases of drug development into a single trial. This is of
paramount interest in small populations, e.g., when developing drugs for rare diseases. Though the sample size is limited still an appropriate dose has to be found and sufficient evidence for its efficacy to be demonstrated. We propose adaptive clinical trial designs with multiple doses and use modelling approaches to (i) establish a positive dose-response profile, (ii) increase the power of declaring effective dose statistically significant, and (iii) support dose selection at an adaptive interim analysis. We extend MCP-Mod methodology to adaptive two-stage designs by using the closed-testing principle and applying an adaptive combination test to each intersection hypothesis. Combining the data from both stages in adaptive confirmatory designs allow for flexible interim decisions based on all (interim) data available of the ongoing trial while always ensuring strict type I error control. In particular, the MCP-Mod approach can be used to obtain model-based dose effect estimates at interim to guide early futility stopping and/or re-design the second stage (e.g. choice of doses, sample size, allocation ratio) and analysis (e.g., dropping of inadequate response models). By the means of clinical trial simulations we show the operating characteristics (e.g., power for PoC or individual dose-control comparison, bias of effect estimates) for specific adaptations rules.

141 Oat Belian: Identification of concoction used by Belian (Indigenous Healer) as medication in the practice of traditional medicine in Lombok Island, West Nusa Tenggara, Indonesia

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A total of 30.4% the proportion of households in Indonesia using traditional health services in 2013. Other survey in 2012 claimed 27% of West Nusa Tenggara (WNT) people are using traditional medicine. The limitations of conventional drugs, rasi factor (potent), cheap, as well as traditions and beliefs are several reasons people using traditional medicine. Concoction of herbs is one of traditional medicine is commonly used by a Belian (indigenous healer). The data is part of the research findings that carried out between 2013-2014 in Sasak people in WNT, Indonesia. The study was conducted using qualitative methods, interviews, participant observation and photo documentation. Sasak community recognizes many types of Belian (indigenous healers) with various specialties they are “general” Belian (treating various diseases), Belian ranak (indigenous midwife), Belian polak / tolang (bone setter), and also Belian Pijat/Urut (masseur). In the treatment process, the Belian uses four kinds of medicines, the Bubus, Minyak, Sembek, and Aik Putek. These concoctions are mixed using natural materials. Bubus is using paddy/rice, while Minyak using the coconut as main ingredient. Usually the ingredients mix with ragi belek (complete local seasonings). Sembek concoction made from Lekok/ Daun Sirih (Piper Betel Leaves), Buak / Pinang (Betel Nut) and Kapur Sirih (Milk of Lime). The last Sasak medicine is Aik Putek (spelled Water). While Aik Putek, Bubus and Minyak are used as both oral and external medication, Sembek is for external used only. The indigenous healers believe that these concoctions can cure many kinds of illness. In conclusion, although biomedicine exists in this area, people still come to Belian for treatment. There
are four traditional medicines commonly used by Belian to treat their clients, namely Bubus, Minyak, Sembek, and Aik. The concoction is using certain ingredients and blended with other ingredients that can be a medication to cure illness.

142 Medical Pluralism in West Nusa Tenggara, Indonesia

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Coexistence of multiple medical traditions is a common phenomenon throughout the world. There are some different medical traditions intertwined, biomedicine, indigenous medicine and alternative medicine. To examine on what terms the medical systems cooperate and compete one another and how these medical providers apply and justify their treatment methods, I had conducted a qualitative study by interviewing 35 indigenous healers, 16 health officials and three alternative therapists in WNT, Indonesia. In the fieldwork I found types of indigenous healers with different specialties such as bonesetter, traditional birth attendant (TBA), masseur, children and cancer healer. Biomedicine is fully controlled and regulated by the government. The health offices in this province are given the responsibility to organize and plan regional health programs. It includes regulating health official staffs and health care facilities in the area. Alternative medicines can be found a few types of therapy that includes Islamic based therapy, cupping and exorcism, acupressure and chiropractic, as well as herbal medicine. In collaboration with other medical systems in particular biomedicine, only TBA that has a partnership program with the official midwife. Competition process can be observed when the government applies traditional treatment in public health centers and alternative methods in hospitals. Alternative medicine also presents a treatment technology options that are not offered by biomedicine and traditional medicine. Conflict occurs when the indigenous healer dealing with health official. Resistance intervention and refusal to cooperate come about due to a lack of communication between practitioners. In conclusion, coexistence of medical systems is ideal situation since holding the same goal in improving public health. The most important thing is how they work together and build communication to achieve the goal without reducing the role of other medical systems.

143 Characteristics of clinical studies of sanfu acupoint herbal patching: a bibliometric analysis

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Neurotransmitter transporters are found on the presynaptic neurons and on glial cells. The function of these transporters is termination of neurotransmission by rapid removal of neurotransmitter molecules from the synaptic cleft. These transporters couple substrate transport to the ion gradients of sodium and
chloride. Structural studies often require the transporter to be removed from its physiological membrane, which can affect its structure or conformation. Crystal structures of the bacterial homolog LeuT (SLC6 family) were solved in three states of the transport cycle: occluded, outward and inward. The recent inward facing structure shows a conformation where the first helix (TM1A) did not seem to be compatible with the membrane environment. We carried out molecular dynamics simulations of LeuT in membrane and micelle environment to investigate the conformational behaviour of TM1A and combined the investigation with distance measurements using LRET. We used POPC as membrane lipids, and build the micelle systems with three different protein-detergent ratios (1:120, 1:140, or 1:160) using the detergent n-Octyl-β-D-Glucopyranoside (BOG) molecules. We observed a rigid body motion of the TM1A helix: it moves out of the hydrophobic core of the membrane. In contrary, TM1A was stable in its position in the micelle simulations. We confirmed this observation by distance measurements of solubilized LeuT in micelles and reconstituted POPC liposomes. This study suggests that the polar part of helix TM1A would not protrude into the membrane core. To further characterize the conformation of the TM1A helix, we used SMD simulations and pulled this helix relative to the scaffold or the core domain. The free energy profile was in line with our findings in the position of TM1A. This study indicates that changes in the environment can affect the equilibrium conformation of LeuT.

144 Scientific evaluation of patient safety measures with two different methods at the Department of Pediatrics and Adolescent Medicine, Division for Pediatric Nephrology and Gastroenterology

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The aim of the PhD-project is to test the hypothesis that specific interventions have a positive effect on patient safety (h1). This scientific evaluation is a sub-project within a patient safety project at the Department of Pediatrics and Adolescent Medicine, Division for Pediatric Nephrology and Gastroenterology (ward E07) under the supervision of Dr. med. univ. Michael Boehm. Different interventions to improve patient safety will be implemented. While the implementation itself is not part of the PhD thesis, the PhD student will use two different methods to evaluate the success of the implemented interventions: a) Application of the trigger tool method - as described repeatedly in literature (Matlow et al. 2011, BMJ Qual Saf; Kirkendall et al. 2012, Pediatrics; Unbeck et al. 2014 BMC Health Services Research) - to measure triggers of closed patient charts within a period of 2 months before and after the implementation of the patient safety interventions. Triggers are defined parameter, for example loss of weight or abnormal body temperature, which were shown to be associated with adverse events and might thus be used as predictive parameters. b) The second method will be a
standardized survey to identify the state of knowledge from inpatients at the time of discharge, before and after implementation of the interventions over a defined period of 2 months in each case. The primary variable of this PhD-project will be the number of detected triggers per 100 patient admissions, which will be determined by the application of the trigger tool method. The measurable results should allow drawing scientifically based conclusions on the effectiveness of the used interventions within the risk management project.
Oral Session 4 “Cancer Research”, 10.00 - 11.00

Chaired by Michael Grusch and Walter Berger

13  CDK4 and CDK6 cooperate in counteracting the INK4 family of inhibitors during murine leukemogenesis

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Cyclin-dependent kinase (CDK) 4 and 6 are key players mediating G1 progression of the cell cycle. When bound to D-type cyclins they become active and phosphorylate their substrates most importantly the retinoblastoma protein (Rb). High CDK6 levels are frequently observed in human malignancies and CDK4/6 inhibitors show promising efficacy against different types of tumors in clinical trials. INK4 proteins negatively regulate CDK4/6 activity and are frequently inactivated upon transformation. To test the significance of this pathway for tumorigenesis we made use of knock-in mice that express a CDK6 (CDK6 R31C) or CDK4 (CDK4 R24C) mutant insensitive to INK4-mediated inhibition. Mice harboring both mutant alleles developed predominantly hematopoietic and endocrine tumors and showed a drastic reduction in lifespan compared to the individual single mutants. Using BCR-ABL-transformed cells as model system we found that CDK6 R31C causes increased binding of p16INK4a to the remaining wild-type CDK4. Only in the presence of both INK4-insensitive kinases we detected hyper-phosphorylated Rb and accelerated disease onset. The importance of CDK4/6 for tumor formation is also reflected by the emerging success of CDK-inhibitors, such as Palbociclib, which has been shown to significantly prolong progression-free survival of breast-cancer patients and hence has been designated as a breakthrough therapy of the year 2013 by the FDA. Our observations unravel compensatory functions among the INK4 family members and reveal that CDK4 and CDK6 cooperate in hematopoietic tumor development. In the presence of at least one functional INK4 protein, the concomitant overexpression of both CDK4 and CDK6 may be required to overcome the limited phosphorylation of Rb that is inflicted by increased binding of the inhibitor to the remaining wild-type CDK. Our study underlines the importance of simultaneous targeting of CDK4 and CDK6 in hematopoietic tumors in which INK4 proteins are inactivated.

14  Loss of cJun enhances tumor formation in a mouse model of prostate cancer


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Prostate cancer (PCa) is the second most common cancer in men worldwide. The current diagnostic tools in PCa are insufficient to distinguish whether tumors remain non-aggressive or develop into metastasizing lethal disease. The lack of markers stratifying low and high risk PCa patients results in frequent overtreatment leading to severe side effects. The contribution of cJun, an important member of the AP1 transcription factor family, to PCa progression is controversially discussed. Our objective is to investigate whether cJun acts as tumor suppressor or oncogene in the Pten-deficient PCa mouse model. We generated a transgenic PCa mouse model by crossing Pb-Cre4 mice with mice carrying floxed alleles of Pten and/or cJun (ΔPten, ΔcJun) facilitating prostate epithelial specific deletion. We analysed PCa development in ΔPten, ΔcJun mice and compared them to ΔPten and wild type mice at the age of 38 weeks. We characterized the tumors macroscopically and histopathologically and performed gene as well as protein expression analyses in order to study the effects of loss of cJun during PCa development. To prove human relevance of our findings, we analysed large cohorts of human patient samples for cJun mRNA expression levels. Concomitant loss of cJun and Pten leads to unexpected and significantly increased PCa tumor growth compared to ΔPten controls. ΔPten, ΔcJun tumors show increased proliferation and decreased apoptosis. Furthermore, ΔPten, ΔcJun tumors revealed a complete bypass of the senescence response with significantly reduced levels of p16, p19 and p53. We identified cJun as an important tumor suppressor in mouse PCa. Furthermore, loss of cJun mRNA and protein expression correlates with poor prognosis in large cohorts of human PCa patients. Loss of cJun could therefore represent a novel marker to stratify high and low-risk PCa patients which may be an important tool to guide therapeutic decisions.

15 Tumor cell - fibroblast interaction influences macrophage recruitment and polarization in colon cancer

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Carcinogenesis is influenced by the tumor microenvironment, which consists of cancer associated fibroblasts (CAFs), endothelial cells and inflammatory cells that are embedded in the extracellular matrix (ECM). CAFs represent the major cellular component of the tumor microenvironment and play a key role in cancer initiation and progression. Here we focused on colorectal cancer (CRC), which is the third most common cancer in western countries. Colon carcinomas are infiltrated by monocytes and tumor associated macrophages (TAMs), which represent the main immune cell component of the tumor
stroma. The recruitment and activation of these cells at tumor sites is thought to be regulated either by tumor- or stromal-derived signals including growth factors and cytokines. Depending on stimuli from the microenvironment, macrophages can be polarized into the anti-inflammatory M2 phenotype. TAMs are mainly of the M2 phenotype and seem to actively promote tumor growth, progression, angiogenesis and tissue remodeling. However, whether the crosstalk of colon cancer cells with CAFs and macrophages can modulate recruitment and polarization is not known in detail so far. Here we show that the interplay of tumor cells and CAFs influences macrophage polarization. We analyzed in a modular experimental setup using individual mono-, co- or triple-cultures, if tumor - stroma crosstalk has an impact on tumor cell invasion, monocyte differentiation and macrophage polarization. Macrophage phenotypes were determined by immunohistochemical and immunofluorescence staining of organotypic co-cultures, flow cytometry, Western blotting and RT qPCR. Results of these experiments will be shown. Further, we demonstrate that monocyte/macrophage recruitment is differentially regulated by tumor stroma interaction in 3D. In addition we analyzed conditioned media for cytokine levels using antibody arrays and found several interestingly regulated molecules, which warrant further functional studies.

16 Correlation of circular RNA abundance with proliferation - exemplified with human normal, benign, and malignant tissues


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Background: Circular RNAs are a recently (re-)discovered abundant RNA species with presumed function as miRNA sponges, thus part of the competing endogenous RNA network. Some support proposes that they origin from specific back-splicing events guided by specific sequences around splice sites. However, neither their formation mechanism nor their cellular function is completely understood. Material and methods: We analyzed the expression of circular and linear RNAs and proliferation in 31 matched normal colon mucosa and tumor tissues. Additionally, immunohistochemical staining of the proliferation marker Ki-67 was performed with the corresponding formalin-fixed paraffin-embedded tissues. The correlation of global circular RNA abundance (the circRNA index) and proliferation was validated in additional human healthy and diseased (benign and malignant) tissues. Results: We predicted >1800 circular RNAs and proved the existence of five randomly chosen examples using RT-qPCR. Interestingly, the ratio of circular to linear RNA isoforms was always lower in tumor compared to normal colon samples and even lower in colorectal cancer cell lines. Furthermore, this ratio correlated negatively with the proliferation index. The correlation of the circRNA index and proliferation was validated in a non-cancerous proliferative disease, idiopathic pulmonary fibrosis, ovarian cancer cells compared to cultured normal ovarian epithelial cells, and 13 normal human tissues. Conclusion: We are the first to report a global reduction of circular RNA abundance in colorectal cancer cell lines and cancer compared to normal
tissues and discovered a negative correlation of global circular RNA abundance and proliferation. This negative correlation seems to be a general principle in human tissues as validated with three different settings. Finally, we present a simple model how circular RNAs could accumulate in non-proliferating cells.
Poster Session “Vascular Biology, Cardiology & Pulmonology”, 13.00 - 15.00
Chaired by Brigitte Hantusch, Peter Schellongowski and Diana Bonderman

145 CD4+CD28null T cells are enriched at the culprit lesion site in STE-ACS and promote NET production
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Background ST-elevation acute coronary syndrome (STE-ACS) is among a leading cause of death. Acute coronary atherothrombosis as the underlying event is still poorly understood. We hypothesized that circulating leukocytes adhere to atherosclerotic plaques and mediate thrombotic occlusion. It has been shown that circulating CD4+CD28null T cells, which release high levels of granzyme B and perforin, are increased in STE-ACS, especially in patients suffering from diabetes and/or recurrent cardiovascular events. Neutrophil extracellular traps (NETs) released by activated polymorphonuclear neutrophils (PMNs) have been shown to be a crucial component in thrombogenesis. We characterized CD4+CD28null T cells at the culprit lesion site in STE-ACS patients and tested their impact on NET formation. Methods We included 150 STE-ACS patients who underwent primary percutaneous coronary intervention. Culprit site blood and solid thrombus material were collected during thrombectomy. In parallel, a blood sample from the femoral arterial sheath was collected. We measured CD4+CD28null T cells in whole blood and solid thrombus specimens. Granzyme B and perforin levels were determined. Isolated PMNs were stimulated with granzyme B and/or PMA, and NET formation was assessed by immunohistochemistry. Results CD4+CD28null T cells were increased at the culprit lesion site both in coronary whole blood and the solid thrombus, compared with peripheral blood (n=106, p<0.0001, 7.79±9.68 vs. 9.92±11.44% of CD4+ cells; n=20, p<0.01, 8.14±10.08 vs. 13.6±14.12% of CD4+ cells). Perforin and granzyme B were decreased in coronary CD4+CD28null T cells and correlated inversely with granzyme B levels in culprit site plasma. Granzyme B induced netosis of PMNs in vitro. Conclusion Granzyme B/Perforin-releasing CD4+CD28null T cells accumulate at the culprit lesion site in STE-ACS, and may directly induce NETosis.

146 CD14+CD16++CX3CR1+ monocytes are increased at the culprit lesion site of STE-ACS patients and protect from myocardial necrosis
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Background ST-elevation acute coronary syndrome (STE-ACS) is the leading cause of death. Mechanisms of coronary occlusion are poorly understood. Monocytes are early inflammatory cells implicated in the
Monocyte subsets are divided according to their CD14:CD16 expression profile into CD14++CD16-, CD14++CD16+ and CD14+CD16++ monocytes. Especially CD14+CD16++ have been shown to play a role in tissue repair. We examined monocyte subset levels and major activation markers at the culprit lesion site (CLS) of STE-ACS patients. Furthermore, we correlated these data with enzymatic infarct size. Methods STE-ACS patients who underwent primary percutaneous coronary intervention at the Vienna General Hospital were consented (n=94). Culprit site blood was aspirated with a thrombectomy catheter and particulate thrombus material was separated. In parallel, blood was sampled from the femoral arterial sheath. Flow cytometry was employed to classify monocytes by their CD14:CD16 ratio, major activation markers and monocyte platelet aggregates. CKMB AUC was calculated using a trapezoidal formula. Data are expressed as median [IQR]. Results Overall, monocytes are significantly decreased at the CLS compared to femoral blood. Monocyte subsets are substantially shifted at the CLS with increased levels of CD16+ subsets (CD14++CD16- femoral 92.37% [87.35-94.5] vs. CLS 89.06% [82.75-93.3], CD14++CD16+ femoral 3.92% [2.58-7.68] vs. CLS 4.82% [2.91-8.47], CD14+CD16++ femoral 3.34% [2.58-7.68] vs. CLS 4.75% [2.29-8.25], all p<0.0001). Increased platelet aggregation with CD16+ monocyte at the CLS could be found. Activation markers are significantly different as CX3CR1 is higher expressed at the CLS, while HLADR was lower. Interestingly, CX3CR1 expression of CD16+ monocytes was negatively correlated with enzymatic infarct size. Conclusion CD14+CD16++ monocytes with enhanced fractalkine-dependent migratory potential protect from myocardial necrosis.

147 Arterial-ventricular coupling in preterm neonates

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Background & Aim: The model arterial-ventricular coupling (AVC) describes the interaction of the left ventricle with the arterial system. Aim was to apply the model AVC and its components (E[downA]) and (E[downES]) in sick preterms with either pulmonary hypertension (PH-group) or hemodynamically significant patent ductus arteriosus (hPDA-group) and in stable preterms with uncomplicated postnatal course (control-group). Patients & Methods: Systolic blood pressure as well as the echocardiographic parameters endsystolic volume and stroke volume for estimating AVC, E[downES] and E[downA] were collected retrospectively. Echocardiographic examinations were performed prospectively in order to
create a register. Selected time points to implicate transitional phase after birth were set from day 1-3, 4-7 and 8-30. Patient recruitment criteria were as follows: preterm birth from 23+0 to 32+6 weeks+days; normal intracardiac anatomy; PH-group: anti-PH treatment due to (supra-) systemic pulmonary pressure; hPDA-group: presence of PDA with an enddiastolic maximal velocity in the left pulmonary artery (LPAdia) \( \geq 0.2 \text{m/s} \); control-group: neither anti-PH treatment nor catecholamines, in the presence of PDA LPAdia < 0.2m/s and a ratio of the left atrium/aorta < 1.4. Results: 36 examinations were included to the PH-group, 42 to the hPDA-group and 89 to the control-group. Time trend analyses showed significant lower AVC in the PH- and hPDA-group compared to the control-group (p=0.05). \text{E}[\text{downES}] \text{was higher in the PH-group (p=0.007) and both E}[\text{downA}] \text{and E}[\text{downES}] \text{were lower in the hPDA-group (E}[\text{downA}]: p=0.0002; E}[\text{downES}]: p=0.02). Conclusion: Higher \text{E}[\text{downES}] \text{in PH results from interventricular interdependence with decreased left ventricular filling. Lower \text{E}[\text{downES}] \text{and E}[\text{downA}] in PDA result from left ventricular overload and systemic steal-effect. Applying the AVC-model may facilitate explaining preterm hemodynamics.}

148  **Vascular morphogenesis in the context of inflammation: continuum meets entity**

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Inflammatory processes occurring during injury and ischaemic diseases are accompanied by neovascularization through both angiogenesis and vasculogenesis. There are few in vitro models addressing these two mechanisms of neovessel formation in association with inflammation. Using a 3D ex vivo explant culture model we investigated the process of vascular morphogenesis in the context of inflammation. Synovial tissue fragments from patients with inflammatory osteoarthritis were cultivated within fibrin matrices for three weeks, and the phenotypic expression pattern of newly formed vascular structures growing into the scaffold was analyzed by confocal laser-scanning microscopy of intact 3D cultures. Leukocyte egress from synovial tissues into the primarily acellular fibrin matrices preceded the appearance of immature sprouts originating from both the embedded samples and from clusters locally formed by tissue-derived emigrated cells. Sprouts simultaneously expressed stem cell-, endothelial cell-, pericyte and hematopoietic lineage markers, and during vessel maturation progressively restricted their phenotype towards a specific identity, e.g., CD34+/CD31+ endothelial cells were abluminally covered by alpha-SMA+ pericytes and type IV collagen-lined lumina contained CD45+ leukocytes. The mesenchymal stem cell marker STRO-1 was consistently detected in association with developing vessels. Samples with
minor inflammatory infiltration showed no vascular outgrowth. In conclusion, in this model neovascularization seems to be actively driven by inflammatory cells and occurs through angiogenesis and vasculogenesis originating from cell clusters. Initially immature vessel-phenotypes exist in a continuum coexpressing stem cell markers together with a variety of typically lineage-restricted markers. Structural maturation of vascular sprouts is accompanied by progressive specialization of vascular cells leading to segregation into lineage marker restricted cellular entities.

Detection of ventricular fibrillation thresholds at an isolated, rabbit heart model

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BACKGROUND: Current research in the area of ventricular fibrillation explains rise of lethal arrhythmias, by electric fields eliciting a change of transmembrane potential, which especially in the repolarization phase causes "Spiral Wave Reentry". This leads to self-sustaining multiple tissue stimulating circulations resulting in an asynchrony of electrical activity in the myocardial fibers and a drastic reduction of cardiac output. The main knowledge about electrophathology of ventricular fibrillation is based on a few publications from the late 80's without any systematic study about ventricular fibrillation at higher frequencies (>1kHz). AIM OF STUDY: Gaining knowledge about the mechanisms and probability of electricity induced ventricular fibrillation at frequencies unequal 50Hz. The further interest lies in the origin and process of ventricular fibrillation (Control of the Spiral-Wave-Reentry Theory), and to adapt the latest knowledge of hazard potential of the electricity related to daily used electrical technology. Based on experiments at the isolated heart, physiological conclusions and disjunct theoretical rudiments, we are trying to find average statements about the presumption of electricity-induced ventricular fibrillation. METHODS: The experiments are performed with female adult New Zealand White rabbits (2500 +/- 200g) and an isolated, erythrocyte-perfused, rabbit working-heart model. After excision, aorta and left atrium of the heart is canulated and perfused in the retrograde Langendorff mode (LD). The measurements are performed in the following anterograde Working Heart (WH) mode. Using an impedance related signal generator, electric stimuli of different wavelengths will be applied to the heart. During the whole experiment ECG and left ventricular pressure are monitored in order to detect ventricular fibrillation.
Anticoagulation assessment with prothrombin time and anti-Xa assays in real-world patients on treatment with rivaroxaban

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Background: Monitoring of anticoagulation with the direct factor Xa inhibitor rivaroxaban is considered unnecessary in a routine clinical setting. However, assessment of its anticoagulant effect may be desirable in certain clinical situations. Methods: We assessed prothrombin time (PT) reagents and commercially available anti-Xa assays (Biophen) calibrated for rivaroxaban and heparin in comparison to liquid-chromatography mass-spectrometry (LC-MS/MS) measurements of rivaroxaban concentration in samples from patients on treatment with rivaroxaban for stroke prevention in atrial fibrillation. Citrate plasma samples were obtained from 30 randomly selected patients on uninterrupted treatment with rivaroxaban for a minimum of 1 month. The anti-Xa assays, direct Xa inhibitor (DiXa-I) and Heparin LRT were conducted for both wide and low calibrations for rivaroxaban. Measurements were compared to LC-MS/MS using correlation, linear regression, intra-class correlation and Bland-Altman analysis. Results: In 30 patients (9 female) of median age 71.5 years and BMI 26.5 kg/m², rivaroxaban concentrations between 2.4 and 625 ng/ml (median 82ng/ml) were measured by LC-MS/MS. PT reagents were poorly sensitive for rivaroxaban concentrations \( r^2=0.52 \) and 0.09). Anti-Xa assays DiXa-I \( r^2=0.95 \) and Heparin LRT \( r^2=0.97 \) were sensitive to rivaroxaban in all concentrations, but especially in low concentrations with low calibrations \( r^2=0.97 \) and 0.98, respectively). The highest agreement occurred between Heparin LRT and low rivaroxaban concentrations with a mean difference of -5.3 ng/ml (limits of agreement -12.9 to 2.4 ng/ml). Conclusion: Anti-Xa assays can indirectly determine the concentration of rivaroxaban for a wide range of concentrations in real-world patients. An interpretation of anti-Xa and PT measurements in treatment with rivaroxaban requires knowledge of the local reagents.

The Angel Valve Concept - 3D Measurements for Construction of a Mitral Valve Device in Mitral Insufficiency Using Commercially Available Software

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Carpentier's French Correction in surgical mitral valve (MV) repair practicing median sternotomy is applied as gold standard for severe mitral regurgitation (MR). Transcatheter mitral valve repair is now considered an innovative alternative. For this purpose we designed a proprietary concept to reestablish mitral sufficiency in most of valve pathologies. To adapt the device to these pathologies the MV is constructed 3D supported by commercially available software. Technology: For assessment of the individual geometry of the MV, a transesophageal echocardiogram (TEE) is performed pre-operatively and imported to software. Mitral annulus, anterior and posterior mitral leaflets (AML, PML) and point of coaptation are defined respecting the continuity to aortic valve. Based on these parameters a multiplane reconstruction (MPR) of the MV is computed. If necessary, the closing line of the leaflets can be manually adjusted. Furthermore, position of papillary muscles (PM) for insertion of artificial chords is determined. The distance from the middle of the annulus of PML to the commissures needs to be known for implantation. Results: Prototypes of devices for several pathologies e.g. posterior leaflet prolapse, restrictive leaflet, Barlow’s disease or annulus dilatation are designed. As the wing-like structure consists of a proximal and distal part squeezing PML in between and anchoring it by pressing it against the mitral annulus from above and below, problems with fixing the device are avoided. Moreover, it still allows the natural PML some limited motion while AML remains structurally and functionally intact thus ensuring satisfying coaptation plane. Conclusion: Today’s commercially available 3D measurements do not completely determine the size of the device. Hence, amendment of software (supported by TomTec) is necessary to obtain information that can be used to design a personally adapted device serving the purpose of an individual correction of mitral insufficiency.

152 Washout of the left ventricle during LVAD support and effectiveness of intermittent speed changes

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Aim: The natural blood flow pathway in the left ventricle is completely altered by left ventricular assist devices (LVADs). In full support the aortic valve even remains closed during the whole cardiac cycle and these alterations may introduce potential areas of stagnation and formation of thrombi. The influence of periodical intermittent pump speed changes (washout cycle) on intraventricular flow patterns was the aim of this work. Methods: An LVAD assisted transparent heart model was developed and combined with bioprosthetic porcine heart valves to maintain the physiologic unidirectional flow. Three pump speeds were tested combined with/without a washout cycle (2sec:-200 rpms, 1sec: +200 rpms, 60sec baseline speed) to mimic a broad range of clinical situations. Visualization of intraventricular flow was performed with Particle Image Velocimetry (PIV) and a stagnation index (SI) calculated. Results: Setting
stroke volumes (SV) and pump speeds ([omega]) at a constant cardiac output of 5 l/min at a mean arterial pressure of 80 mmHg created hemodynamics similar to clinically observed ones. Higher LVAD speed and ventricular support resulted in higher SI starting from 1.18 s in the partial support situation (SV 27 ml, [omega] 2500 rpm), to 1.38 s in the partial support situation (SV 39 ml, [omega] 2700 rpm) and finally 1.53 s in the full support situation (SV 50 ml, [omega] 2900 rpm). Activation of the washout cycle hardly influenced the flow patterns and did not show any positive or negative effect on the SI, whereas the mean flow remained unchanged. Conclusion: LVADs strongly influence intra-ventricular flow patterns and alter the natural pathway completely. The washout cycle as a method to improve washout of the left ventricle showed some slight alterations but an overall positive or negative performance on the washout of the heart could not be proven in the stagnation index.

153 3D direct co-culture model of endothelium on smooth muscle cells to study Neo-intimal hyperplasia of vascular grafts

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Myocardial infarction is caused primarily by occlusion of coronary arteries. Bypass surgery is one among the possibilities to reconstitute perfusion of the infarcted area. For this purpose, either vein or arterial grafts are used. However, their long term patency rates are poor and correlate with a phenomenon called neo-intimal hyperplasia. This is the result of pathological smooth muscle cell proliferation and migration, which narrows the lumen and is accompanied by inflammation. This is initiated very early after the grafting procedure, but the causal molecular mechanisms are yet poorly understood. Hence, we plan to elucidate the initial molecular events involved in endothelial cell activation and subsequent smooth muscle cell proliferation. For this purpose we have established an in vivo grafting model, an organ culture model and an in vitro three-dimensional (3D) co-culture system consisting of a monolayer of endothelial cells growing on top of quiescent smooth muscle cells. We have validated these systems by comparative analysis of expression and time kinetics of E-selectin, ICAM-1, VCAM in response to TNF by FACS, RT-PCR, IF, confocal LSM and western blotting. We also have analyzed the proper development of a basal lamina typically consisting of collagen IV and laminin between endothelium and smooth muscle cells in the co-culture. Future experiments aim to recapitulate neo-intimal hyperplasia as seen in vessel grafts in vivo in this newly established 3-D in vitro model and to identify ways to interfere with the pathology of neo-intimal hyperplasia.

154 Prevention of Neo-intimal hyperplasia in vascular grafts

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In case of vascular occlusion there are several possibilities to restore blood circulation. One of these techniques is bypass surgery. The concept of this technique is to bridge the occluded area with a healthy blood vessel to enable unhindered circulation. The drawback is that vascular grafts are prone to vascular occlusion for which neointimal hyperplasia is a predisposing risk factor. The general appearance of a non-diseased blood vessel is that the inner lining is covered by endothelial cells which are in constant contact to the bloodstream. They form a selective monolayer between tissue and circulation and are therefore a vital component of healthy blood vessel architecture. Endothelial cells sit on a membrane called intima, which separate endothelial cells from underlying smooth muscle cells. The hallmarks of neointimal hyperplasia are a proliferative burst of smooth muscle cells paired with a migratory phenotype so that the intima is no longer separating smooth muscle cells from endothelial cells. The proliferative burst also causes inflammation, which ultimately results in restenosis and occlusion of the vessel. The aim of this study is to elucidate the molecular mechanisms which trigger neointimal hyperplasia. I have established an in vivo model mimicking bypass surgery in humans. I have analysed graft activation at time points before surgery and 1, 6, and 24h post grafting by isolating mRNA and subsequent RNAseq analysis. Results are used (1) to characterize initial events after surgery and (2) to therapeutically interfere with this process in order to reduce risk for restenosis.

155 Differentiation of murine haematopoietic stem cells into megakaryocytes and their functional characterisation

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The maturation and differentiation of haematopoietic stem cells into megakaryocytes are tightly orchestrated processes, which are crucial for the generation of platelets. They increase in size, become polyploid, form a complex membrane system and extend proplatelets into the blood stream. Ploidy represents a marker for general maturity of megakaryocytes. Chemotaxis is crucial for the successful release of platelets into the blood stream as megakaryocytes have to migrate to sinusoidal capillaries. Understanding imbalances in these processes could provide further insights in thrombus formation and has implications for cardiovascular diseases. We isolate haematopoietic stem cells from mouse femora and differentiate them into megakaryocytes in the presence of thrombopoietin. Polyploidy is determined by nuclear staining and analysis by flow cytometry. Their capability to migrate to vessels is observed via a stromal derived factor 1-α (SDF1-α) chemotaxis assay. To assess their potential to differentiate into platelets, mature cells are seeded onto fibrinogen for 6 hours and morphologically categorised via confocal microscopy. We can demonstrate that our method leads to reproducible differentiation of megakaryocytes within a week. We show and quantify several ways of how platelets can be released in
vitro. Combined with polyploidy and chemotaxis assays we provide important techniques to determine the fate of megakaryocytes. These could serve as methods to assess potential platelet function disorders. The presented assays cover each step of platelet release and provide the basis to investigate the impact of genes, pharmacological intervention or environmental factors on megakaryocyte maturation and/or differentiation. We will employ these methods to investigate the impact of chronic inflammation on megakaryocyte differentiation and maturation.

156  **Platelet activation at the onset of human endotoxemia is undetectable in vivo due to rapid platelet clearance**

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Platelets are essential in both hemostasis and immune responses. Platelet activation upon infection enhances microvascular thrombosis, thus affecting microcirculation and promoting disseminated intravascular coagulation. Endotoxemia is well known to have pro-inflammatory and pro-coagulatory effects that enhance platelet activation. However, the immediate direct effects of endotoxemia on platelets have not been studied yet. Thus, we aimed to determine the early effects of the endotoxin lipopolysaccharide (LPS) on platelet function in vivo. In a human endotoxemia model healthy volunteers (n=15) were challenged with LPS (2 ng/kg). Blood was drawn before, 10, 30 and 60 min after LPS injection and platelet activation was determined by flow cytometry (surface CD62P and CD40L, GPIIb/IIa activation, reactive oxygen species formation and platelet-leukocyte aggregates) and ELISA (sCD62P, sCD40L and CXCL4). Further, whole blood and platelets were stimulated in vitro with LPS (50 pg/mL) and analyzed for the same platelet activation markers and for their adhesion to HUVECs under flow. In vitro LPS stimulation increased platelet-leukocyte aggregates and activated platelets within 10-30 min in both whole blood and platelets, thus demonstrating that the presence of leukocytes is not required for LPS-induced platelet activation. In contrast, in vivo challenge with LPS only raised platelet CD40L, but also led to elevated plasma CXCL4 levels within 10 min. However, in vivo LPS challenge also led to a significant decrease in platelet count which could be due to enhanced adhesion to the microvascular endothelium. In line with this hypothesis, in vitro LPS stimulated platelets show a tendency for increased adhesion to HUVECs under flow. In conclusion, LPS rapidly triggers platelet activation and degranulation. However, these platelets are quickly scavenged and thus no longer detected in the circulation. This might explain the drop in platelet count observed at the onset of endotoxemia.

157  **Abdominal aortic aneurysm formation in Tenascin-C deficient mice**

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BACKGROUND: Tenascin-C (TNC) is a matricellular protein produced by vascular smooth muscle cells and fibroblasts in various remodeling processes. In numerous cardiovascular pathologies high TNC levels are associated with unfavorable outcomes. TNC production has also been found in abdominal aortic aneurysms (AAA). The aim of the study is to evaluate whether TNC deficiency could attenuate AAA formation.

METHODS: We compared male AJ TNC -/- and AJ wildtype (WT) mice. After laparotomy and preparation of the infrarenal aorta, AAA were induced by periaortal CaCl$_2$ 0.5M application for 15 minutes. In the sham-operated groups the same procedure was performed, however aortas were incubated with saline solution. The aortic diameter was measured during the procedure and 3 weeks after AAA induction. The main parameter was the ratio of the diameters.

RESULTS: TNC knockout (KO) mice with AAA showed significantly lower diameter ratios than the wildtype group (TNC KO: 1.39±0.25, WT: 1.67±0.22 p<0.05). No significant changes in diameter ratios were found in sham groups (TNC KO: 0.92±0.08, WT: 0.96±0.22, n.s.).

CONCLUSIONS: In our study we found first evidence that TNC deficiency is associated with reduced AAA formation. To evaluate the influence of TNC on long-term AAA development we are currently investigating the AAA progression 10 weeks after induction. To identify possible causal pathways immunohistological and molecular biological assessments will be conducted.

Deciphering the regenerative potential of PICS0 on the excised as well as the implanted heart when applied on heart transplant patients before excision - Evaluation of the study protocol

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Background: The pressure-controlled intermittent coronary sinus occlusion (PICS0) is a catheter intervention temporarily increasing coronary venous pressure. Systolic pressure peaks activate vascular cells in cardiac veins. On a molecular basis PICS0 initiates salvage pathways due to IL-6 and BNP secretion, and neoangiogenesis in myocardial infarct zones. Besides myocardial salvage, PICS0 causes an upregulation of vasoactive as well as cardioprotective molecules, which influence necroapoptosis in ischemia and reperfusion. According to our hypothesis “embryonic recall” PICS0 initiates regenerative pathways via soluble factors exuded by hemodynamic force. The objective of this study is to evaluate whether PICS0 has the ability to activate regenerative mechanisms in cardiomyocytes in failing hearts. The procedure will be applied before the excision of the recipient’s heart during heart transplantation to demonstrate the potential effects of PICS0 in treated myocardium. Furthermore clinical follow up in transplanted patients should decipher whether soluble factors may influence the transplanted donor heart as well by improving functional performance. The method used in this study includes blood sampling from arterial and coronary sinus as well as tissue sampling from the explanted heart, which will be analyzed for salvage pathways. These parameters will be related to functional ones in the transplanted patient. This includes a modified Minnesota quality of Life questionnaire as well as other routine parameters. So far in the heart transplant data base there have been ten patients screened, who have been enclosed and await randomization. As Results we expect according to previous experimental
and clinical observations that PICS0 is able to activate regenerative mechanisms in the explanted heart and eventually may improve cardiac performance and quality of life as well as prognosis of survival in heart transplant patients via soluble factors integrated in the donor heart.

159 Fibrocytes accumulate at the culprit lesion site and display enhanced migratory and reparatory properties in ST elevation acute coronary syndrome

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INTRODUCTION: ST-elevation acute coronary syndrome (STE-ACS) is a major cause of death. Fibrocytes, a Collagen-I+CD34+CD45+ progenitor cell population, are increased in ischemic myocardium of patients. This finding was also observed in a mouse model of ischemia/reperfusion cardiomyopathy. In ACS patients, circulating fibrocytes were shown to be decreased compared to stable angina and healthy controls. We hypothesized that fibrocytes are increased, more active and more susceptible to mitogenic signals within the coronary vessels. This might contribute to occlusion and consecutive reparative processes. METHODS: Blood samples from the coronary culprit lesion site (CLS) of STE-ACS patients (n=50) drawn in the course of primary percutaneous coronary intervention were analyzed. Blood from the femoral artery served as a peripheral control. Flow cytometry was employed to characterize fibrocytes based on their expression of cell-surface marker expression. SUMMARY: Fibrocyte count is increased at the coronary site compared to femoral blood. Furthermore, CLS fibrocytes display significantly increased expression of Collagen-I. The adhesion markers CD11b and CD13 are upregulated in CLS fibrocytes compared to femoral fibrocytes. However, in patients suffering from dyslipidemia, BMPRII expression of fibrocytes was significantly upregulated at the femoral site. CONCLUSION: The increase of CLS fibrocyte count compared to femoral blood is possibly due to homing to the coronary vessels in STE-ACS. This might be mediated by the upregulation of the adhesion markers CD11b and CD13 on CLS fibrocytes. Increased Collagen-I expression of fibrocytes at the CLS might reflect an increased reparative activity of fibrocytes within the coronary vessels. A general pro-inflammatory state associated with dyslipidemia might induce an upregulation of BMPRII expression of fibrocytes. This might increase the susceptibility of fibrocytes to signaling via the bone morphogenic protein-family.

160 Transendothelial migration dependent on transforming growth factor beta in hepatocellular carcinoma progression

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Transendothelial migration of malignant hepatocytes is required for the entry into vasculature, a process termed intravasation. Mechanism of intravasation is poorly understood due to lack of appropriate study tools. We established a model of transendothelial migration by employing malignant hepatocytes which have undergone epithelial to mesenchymal transition (EMT) dependent on transforming growth factor (TGF) beta and liver sinusoidal endothelial cells. The aim was to study the molecular mechanisms of individual cell movement of EMT-transformed hepatocytes through hepatic sinusoidal blood endothelial barriers. Murine liver sinusoidal endothelial cells (mLSECs) were isolated by liver perfusion from p19ARF-/- mice. Functional properties of LSECs were assessed by tube formation and response to anti-angiogenic compounds. Integrity and polarity of endothelial cell layers were determined by transendothelial electrical resistance and confocal immunofluorescence analysis, respectively. Proteome profiling using stable isotope labeling with amino acids (SILAC) was performed to analyze real-time changes in protein expression during transendothelial migration. Lack of p19ARF allows immortalization of genetically stable LSECs expressing the endothelial markers VEGFR-2, CD31 and VE-cadherin. LSECs show VEGF-induced tube formation and apoptosis after administration of the VEGFR inhibitor sunitinib. LSECs grow in monolayers by exhibiting endothelial cell-to-cell junctions including cell polarity. SILAC-labeled EMT-transformed hepatocytes were allowed to cross the layer of LSECs that were seeded on the collagen coated Transwell inserts. Expression of SILAC labeled proteins was profiled in kinetics of endothelial transmigration in the presence and absence of TGF-beta. We established a novel cellular model to accurately analyze the individual cell movement and intravasation of EMT-transformed hepatocytes through the liver sinusoidal blood endothelium in a TGF-beta dependent fashion.

161 Parenteral treprostinil induces a phenotypic shift of circulating monocyte subsets

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BACKGROUND AND AIMS: Pulmonary hypertension (PH) is a disease of occlusive pulmonary vascular remodeling. Key histologic features are intimal fibrosis, smooth muscle cell hypertrophy, adventitial fibrosis, vascular occlusion and thrombosis. Increased pulmonary vascular resistance augments right ventricular load and eventually leads to right heart failure. Early vascular changes have been reported to involve vascular inflammation, including mononuclear cells. We investigated monocyte subsets in patients with severe PH, prior to and after initiation of parenteral treprostinil. METHODS: Peripheral blood samples were drawn prior to (baseline), one week and one month after treatment initiation in 10 treatment-naive patients (6 females, age=71±10.1). Monocytes were characterized based on their expression of CD14 and CD16 (CD14++CD16− corresponding to classical, CD14+CD16+ corresponding to intermediate, CD14+CD16++ corresponding to non-classical monocytes), CX3CR1, HLA-DR, TLR-2, TLR-4, IL-6 and BMPR-II. RESULTS: Treprostinil treatment led to a shift of monocyte subsets. Classical monocytes significantly increased, whereas intermediate and non-classical monocytes decreased. The total number of monocytes did not change. The expression of HLA-DR on classical monocytes was significantly upregulated. CONCLUSION: In the present study, classical monocytes were shown to comprise 76.5±7.1% of total monocytes in severe PH at baseline, compared with a fraction of 85-90% of
total monocytes in normal subjects. Because classical monocytes exert phagocytic properties, removal of antigenic debris and thrombus may be delayed in PH, thus enhancing vascular occlusion. Our data illustrate that PH patients have a three-fold higher proportion of intermediate monocytes compared with healthy controls. Because intermediate monocytes exert pro-angiogenic and antigen presenting functions, further experiments will focus on these properties and their involvement in occlusive vascular remodeling.

162 In-vivo and ex-vivo Functional Characterization of LV Remodeling after Myocardial Infarction in Mice


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Aims: The interest in cardiac remodeling (REM) has steadily increased during recent years. The aim of this study was to functionally characterize REM following myocardial infarction (MI) in mice using high-end in-vivo and ex-vivo methods. Methods and results: MI or sham operation was induced in A/J mice. Six weeks later mice underwent cardiac MRI and were subsequently sacrificed for ex-vivo measurements on the isolated heart. Thereafter, hearts were trichrome stained for infarction size calculation. MRI showed significantly reduced ejection fraction (p<0.01) as well as increased end-systolic and end-diastolic volumes (p<0.01) after MI. The mean infarct size was 48.8 ± 6.9 % of left ventricle. In the isolated working heart (WH) coronary flow (timepoint 20': 6.6 ± 0.9 vs. 13.9 ± 1.6 ml/min, p<0.01), cardiac output (timepoint 20': 17.5 ± 2.6 vs. 36.1 ± 4.3 ml/min, p<0.01) and pump function (80mmHg: 2.15 ± 0.88 vs. 4.83 ± 0.76, p<0.05) were significantly attenuated in MI hearts during all measurements. Systolic and diastolic wall stress was significantly elevated in MI animals. Conclusion: This two-step approach is reasonable, since data quality increases while animals are not exposed to major additional interventions. Both the WH and MRI offer a reliable characterization of the functional changes that go along with the development of post MI REM. By combining these two techniques additional information such as wall stress can be evaluated.

163 The new St. Thomas Hospital polarized cardioplegia: improved efficacy of myocardial protection in pigs

Objective: Increasingly, patients undergoing cardiac surgery are more elderly and sicker and hence require improved protection. We compared the cardioprotective efficacy of a new St Thomas Hospital Polarizing cardioplegia (STH-Pol: esmolol, adenosine, magnesium) to conventional St Thomas Hospital cardioplegia (STH2: potassium, magnesium) in a pig model of cardiopulmonary bypass (CPB). Methods: Pigs (47 ± 4 kg) were anesthetized and monitored for baseline hemodynamic function. After sternotomy, CPB and aortic cross-clamping, hearts were arrested via antegrade warm (37°C) STH-Pol (n=7) or STH2 (n=6) for 60 min ischemia followed by 60 min reperfusion. After weaning from CPB, hearts were monitored for a further 120 min before sacrifice and tissue sampling (for high-energy phosphates and electron microscopy). Recovery was measured as % of baseline (mean±SEM). Results: Baseline hemodynamics were comparable. After 180min reperfusion, recovery of mean arterial pressure and heart rate were similar; however, in STH-Pol hearts had improved recovery of left ventricular systolic pressure (133 ± 8 vs 97 ± 5 %, p<0.01) and external heart work (145 ± 16 vs 88 ± 10 %, p<0.05) than STH2 hearts. Coronary flow/heart weight was also higher during early (430 ± 59 vs 211 ± 59 %, p<0.05) and late reperfusion (269 ± 43 vs 90 ± 16 %, p<0.01) in STH-Pol. Total CK release was lower in STH-Pol hearts during reperfusion (2016 ± 262 vs 1232 ± 199 U/l, p<0.05). Creatine phosphate levels in ST-POL hearts were higher (133 ± 31 vs 63 ± 2 nmol/mg, p<0.05). There was no difference in ultrastructure between groups. Conclusion: Polarized cardiac arrest improved myocardial protection and reduced ischemic damage in a model of CPB in pig hearts. This new polarizing cardioplegia may have beneficial effects for clinical use.

164 Inducing angiogenesis with a trans-coronary sinus catheter intervention

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Background: Besides advances in heart failure therapies, an increasing number of patients cannot benefit from available options. It has been shown that PICSO activates cardio-venous endothelium re-opening
pathways for endogenous repair. The aim of this study was to substantiate angiogenesis induced by PICS0 in an ischemia/reperfusion model. Methods: 32 pigs were divided into 4 groups: a) Pigs with infarct and reperfusion (control-group, n=8), b) pigs with PICS0, ischemia and reperfusion (PICS0-A, n=11), c) pigs with PICS0 without ischemia and reperfusion (PICS0-B, n=10) and d) sham-operated pigs (n=3). LAD was occluded for 3 hours followed by 1 hour reperfusion. PICS0 was induced after 30 minutes ischemia and continued through reperfusion. The heart was divided into 5 areas (BorderA, BorderB, Infarct zone/LAD, Remote, Right ventricle). Results VEGFR1 was significantly upregulated in both border zones in PICS0-A as compared to controls (p<0.05). LAD regions in PICS0-B were significantly upregulated compared to control groups (p<0.05), whereas no significant difference was found in remote or right ventricular areas. Conclusion: Significant upregulation of VEGFR1 proteins by PICS0 in early reperfusion reflects angiogenesis in infarct and border zones. Therefore PICS0 appears to reopen endogenous regenerative pathways leading to an induction of angiogenesis in reperfused myocardium.

165 Preoperative serum creatinine and postoperative outcome in cardiac surgery - Is body composition a confounder


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Introduction: Preoperative renal insufficiency is a predictor for mortality after cardiac surgery and is associated with the development of acute kidney injury (AKI).(1) AKI is diagnosed by the absolute serum creatinine (SCr) level, its change within one week and/or the need for dialysis. SCr is usually produced at a relatively constant rate by the body depending on the amount of muscle mass, and is a reliable surrogate marker for kidney function.(2) In this splitted study we analysed in a retrospective cohort analysis the cut-off values for risk stratification based on SCr above which mortality increases. And further we want to determine prospectively if the incidence of AKI is different in patients below or above the estimated cut-off and if increased bSCr is influenced by body composition. Methods: 9,490 cardiac surgical patients, from 1997 to 2008 (follow-up until 2010) were included. For cut-off identification the data set was split into two groups iteratively for every value between minimum and maximum with a step width of 0.1 mg/dL and the non-parametric Log-Rank statistic was calculated. For the prospective study we will include 200 elective cardiac surgical patients matched in two groups depending on the estimated cut-off resulting from the retrospective analysis. For our primary outcome, SCr will be sampled 7 days postoperative and currently diagnosis pathways for AKI will be used. Results: We found the best cut-off for SCr is >1.3 mg/dL to be predictive for survival. 19.5% were found with increased SCr above its cut-off and associated with an elevated mortality risk. Conclusion: A preoperative SCr cut-off for mortality after cardiac surgery was identified. In the next step the influence on AKI and the influence of body composition on preoperative renal function has to be investigated.
Asphyxia by drowning induces massive bleeding due to hyperfibrinolytic disseminated intravascular coagulation


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Objective: To date no study has systematically investigated the impact of drowning induced asphyxia on haemostasis. Our objective was to test the hypothesis that asphyxia induces bleeding by hyperfibrinolytic disseminated intravascular coagulation (DIC). Design: Observational study. A 2100 bed tertiary care facility in Vienna, Austria. Patients: All cases of drowning induced asphyxia (n=49) were compared to other patients with cardiopulmonary resuscitation (CPR; n=116) and to patients with acute promyelocytic leukemia (APL; n=83). Six drowning victims were investigated prospectively. To study the mechanism, a forearm-ischemia model was used in 20 volunteers to investigate whether hypoxia releases tissue plasminogen activator (t-PA). Interventions: None. Measurements and Main Results: Eighty percent of patients with drowning induced asphyxia developed overt DIC within 24 hours. Compared to non-drowning cardiac arrest (CA) patients, drowning patients had a 13-fold higher prevalence of overt DIC on admission (55% vs. 4%; p<0.001). Despite comparable DIC scores, APL patients had higher fibrinogen but lower d-dimer levels and platelet counts than drowning patients (p<0.001). Drowning victims had a 3-fold longer activated partial thromboplastin time (APTT, 124s; p<0.001) than both non-drowning CA and APL patients. Hyperfibrinolysis was reflected by up to 1000-fold increased d-dimer levels, >55-fold elevated plasmin-antiplasmin levels and a complete absence of thrombelastometric clotting patterns, which was reversed by antifibrinolytics and heparinase. Thirty minutes of forearm-ischemia increased t-PA 31-fold (p<0.001). Conclusions: The vast majority of drowning patients develops overt hyperfibrinolytic DIC, partly caused by hypoxia induced t-PA release. Antifibrinolytics and heparinase partially reverse the abnormal clotting patterns. Severe APTT prolongation may be a marker of combined hyperfibrinolytic afibrinogenemia and auto-heparinization in drowning related asphyxia.

Possible influence of blood flow modulations generated by ventricular assist devices on the endothelial surface layer

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Introduction: Cardiovascular diseases are associated with endothelial dysfunction and microvascular failure. Herein, a shedding of the glycocalyx barrier is anticipated to occur due to altered blood flow patterns and shear stress exposure, which is also induced by ventricular assist devices (VADs).

Methods: Sublingual capillaroscopy using a Sidestream Darkfield videomicroscope was performed in 39 patients with VAD support (diagnosis: predominantly ischemic and dilated cardiomyopathy) and analyses compared with 39 healthy subjects. The perfused boundary region (PBR) was calculated as an indicator of glycocalyx barrier properties by the GlycoCheck measurement software (Glycocheck TM, Maastricht, the Netherlands). Furthermore, coagulation assays (INR, thrombin time, aPTT, Normotest) were performed and the biomarkers C-reactive protein, D-dimer, and fibrinogen measured.

Results: Mean time after VAD implantation was 20.1 ± 14.1 months; patients were treated with the continuous flow VAD-devices HeartWare® (centrifugal flow device, n=27) and HeartMate®II (axial flow device, n=12). In comparison to healthy subjects, patients with VAD support showed an increased PBR (p=0.003) signifying a loss of the endothelial surface layer. There was no difference in PBR between patient subgroups regarding disease etiology and device types, furthermore no correlation between PBR and inflammatory parameters could be observed. Conclusions: These observations suggest the influence of mechanical forces on endothelial glycocalyx modulation. Further studies will be needed to give more insights into the disease patterns occurring in patients with cardiomyopathy.

Human amniotic epithelial cells as potential source for pulmonary surfactant

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Pulmonary surfactant is a surface-active lipoprotein complex produced by alveolar epithelial type 2 cells (AEC2), which reduces surface tension and consequently prevents the collapse of alveoli. Most surfactant protein deficiencies have a poor prognosis and lead amongst others to respiratory distress syndrome (RDS), chronic lung diseases or even lung failure. Currently there is no specific treatment available and surfactant replacement therapy is expensive. Epithelial cells derived from the human amnion (hAECs)
have been proven to be highly promising cells for therapy. They show low immunogenicity and have already been successfully differentiated towards various cell lineages, including AEC2 cells. In this study we aimed to investigate the ability of differentiated hAECs to produce pulmonary surfactant in detail and compare them with native hAECs. For this, hAECs were isolated from human amnion and analyzed for surfactant protein expression either directly after isolation or after culture both in differentiation and control medium via Flow Cytometry and/or immunofluorescent staining. For verifying surfactant secretion ELISA was performed. We could show that differentiated hAECs produced surfactant proteins over several passages without losing stem cell marker expression. Moreover secretion of surfactant protein D (SP-D) into the cell culture supernatant was demonstrated. Interestingly, also native cells, directly after isolation, exhibited expression of surfactant proteins, maintained by culturing in control medium only. According to our study both differentiated as well as native hAECs are able to produce and secrete pulmonary surfactant. This means that hAECs, even in a native state, could represent a promising source for pulmonary surfactant. If it is possible to stimulate the cells to increase the secretion, the resulting conditioned cell culture media could be tested in vivo against RDS and other surfactant-associated diseases.

169 Helicobacter pylori released virulence factors can cause platelet activation

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Background. Helicobacter pylori is a Gram negative microorganism, which can be found in the gastric mucosa and the epithelial cells of the stomach in more than 50% of the human population. The adequate gastric microcirculation helps to maintain an intact stomach tissue. H. pylori in the mucosal layer may get in contact with the gastric microcirculation and may also release bacterial virulence factors into the blood. Aim. We examined the contribution of vacA, with and without plasma proteins to platelet activation. Materials & methods. Citrated blood from healthy donors with known H. pylori infection status was used for platelet isolation by OptiPrep density gradient centrifugation. Washed platelets or platelet rich plasma (10^8 cells) were exposed to 6 x 10^6 CFU/ml vacA positive (ATCC 49503) or vacA negative (ATCC 51932) live bacteria in liquid culture. To examine the effect the released virulence factors, the liquid culture supernatant and a row protein extract from the supernatant was used. Platelets were analyzed by flow cytometry from different timepoints: immediately after exposure to the H. pylori, after 1 hour and after 3 hours. Platelets were stained with anti-CD41-APC, anti-P-selectin and anti-CD63 antibodies. Results, conclusion. Our results indicate that vacA doesn't seem to be the main contributor to platelet activation. There should be an other released virulence factor what is produced independently from vacA, it needs further investigation. In this process, plasma components seem to act as inhibitors, as we have seen the lower level of P-selectin surface expression using platelet rich plasma.

170 Short term and sustained effects of device guided breathing on blood pressure and heart rate variability in hypertensive diabetic patients
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**Poster Session “Cancer Research”, 13.00 - 15.00**

Chaired by Christoph Gasche, Martin Hohenegger, Isabella Ellinger and Katharina Lampichler

171 Oxidative stress is a driver of chromosomal instability and tumorigenesis in IL-10KO mice

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Patients with inflammatory bowel disease like Crohn’s disease (CD) and ulcerative colitis (UC) have a higher risk of developing colitis-associated cancer (CAC); however, the underlying mechanism of disease progression from colitis to CAC is not well understood. IL-10 and IL-10R polymorphism are implicated by GWAS studies in susceptibility for UC. In inflammation-driven carcinogenesis, genetic instability and oncogenic signaling can be driven by perpetual oxidative stress. Here we investigated cellular processes implicated in colon carcinogenesis in a mouse model of CAC. Genetic and epigenetic mechanisms involved in CAC development and progression were investigated: loss of heterozygosity (LOH), microsatellite instability (MSI) and CpG methylator phenotype (CIMP). Fragment analysis of standard mouse markers (Kabarrah et al. Mol Carcinog 2003) and of mouse chromosomes 11 (p53 locus) and 13 (Apc locus) showed that neither MSI nor LOH were present. No change in CIMP was observed upon genome-wide methylation analysis by PCR with primers specific for the murine B1 element. Immunohistochemistry demonstrated strong activation of histone H2AX and Mre11, indicative of double-strand breaks (DSB). Guanine oxidation was increased in epithelial cells as marker of oxidative stress and certain double-strand repair pathways (Brca1 for homologous recombination and Ku70 for error prone non-homologous end-joining) were activated. The level of apoptosis was unchanged indicating that most cells rather undergo DNA repair and survive. These data indicate that inflammation-driven carcinogenesis induces oxidative DNA damage and overt DSBs. The induction of error prone DSB repair without an increase in apoptosis may likely contribute to genomic instability and CAC.

172 SR-BI indicates human prostate cancer progression

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Prostate cancer is among the most common cancers in men worldwide and its prevalence shows a strong association with elevated cholesterol levels in the plasma. Here, we screened prostate cancer samples
for alterations in HDL and LDL receptor amounts. We identified SR-BI mRNA to be up-regulated in prostate cancer samples of high Gleason scores as well as in already metastasized prostate cancer samples. Our analysis revealed that 53.6% of all cancer samples, but none of the non-cancer samples showed high SR-BI protein expression. Additionally, Gleason score equal or higher than 7 was associated with high SR-BI protein expression. Moreover, disease free survival time was reduced (p=0.02) in high SR-BI expressing patients. It has been shown that hormone refractory prostate cancers show elevated mechanistic target of rapamycin signaling and we were able to demonstrate positive correlation of ribosomal S6 Kinase phosphorylation and SR-BI expression. In conclusion we have shown that SR-BI indicates human prostate cancer formation and we speculate that increased levels of SR-BI could be involved in the generation of a castration resistant phenotype.

173  High Accuracy of Soluble Axl in the Differential Diagnosis of Chronic Liver Diseases and Hepatocellular Carcinoma

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Introduction: Diagnosis of hepatocellular carcinoma (HCC) at early stages allows curative therapies, whereas treatment options at later stages are very limited. State-of-the-art diagnosis of HCC by ultrasonography and determination of serum Î±-fetoprotein (AFP) levels shows moderate sensitivity and limited specificity, thus highlighting the need for more accurate biomarkers in the diagnosis of early stage HCC. In this multicenter study we assessed the potential of soluble Axl (sAxl) as a diagnostic biomarker of early HCC and cholangiocellular carcinoma (CCC) as well as examined the value of sAxl in the differential diagnosis between chronic liver diseases (CLDs) and HCC. Methods: Levels of sAxl, a cleavage product of the receptor tyrosine kinase Axl, were analyzed by enzyme-linked immunosorbent assay in 814 serum samples from centers in Europe and China. Results: Analysis of sAxl showed significantly increased levels in HCC as compared to healthy controls. Receiver operating characteristics (ROC) curve analysis revealed high sensitivity and specificity of sAxl in very early stage HCC (BCLC 0) and early HCC (BCLC A) compared to AFP. HCC patients negative for AFP displayed significant sAxl serum levels and combination of sAxl and AFP improved diagnostic accuracy in very early HCC patients. Differential diagnosis revealed high levels of sAxl in HCC versus CLDs derived from non-alcoholic fatty liver disease (NAFLD/NASH), autoimmune hepatitis (AIH), primary sclerosing cholangitis (PSC) and primary biliary cirrhosis (PBC). Furthermore, independent stress testing revealed long-term storage and temperature stability which corroborates the potential of sAxl as valuable biomarker in clinical diagnostics. Conclusion: Assessment of sAxl levels in blood samples allows accurate differential diagnosis of very early HCC versus fibrosis and cirrhosis, suggesting that sAxl is a promising diagnostic biomarker for routine clinical use.

174  Evaluating PI3K/AKT/mTOR and MAPK signaling in 3D colon cancer models to identify effective combination therapy approaches
Conventional preclinical testing of potential anti-cancer compounds relies on drug response of tumor cell lines grown as 2D cell cultures on flat plastic surfaces and as ectopic xenograft models in vivo. These simplified models ignore two major features of malignant solid tumors: 1.) Carcinomas and sarcomas grow as 3D structures in the body and 2.) Cancer cells grow in mutual interaction with non-malignant neighboring human cells - the so called “tumor stroma” which is a major constituent in tumor development and progression. Both, 3D growth and tumor-stroma interaction profoundly alter drug sensitivity and are therefore of utmost importance in preclinical cancer models. We analyzed the PI3K/AKT/mTOR and MAPK pathways, which are the most frequently altered signaling cascades in human cancer, in 2D versus 3D cultures of colon carcinoma cell lines. Indeed, we found altered signaling in multicellular spheroids (3D) compared to conventional 2D cell culture, which correlates with differential cell cycle distribution, cell/spheroid size and cellular viability. We found differences in drug response to PI3K/AKT/mTOR and MAPK inhibitors in 2D vs 3D culture. Analysis of certain drug combinations revealed a prominent induction of apoptosis selectively in 3D. Further, we will integrate paracrine stromal fibroblast effects into the treatment of colon cancer cells in 2D and 3D culture. These promising results qualify the 3D cancer model as a suitable tool for preclinical testing of potential anti-cancer drugs.

Caspase 8 activation mediates hypersensitivity to KP1339-treatment

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The ruthenium-based compound KP1339 (trans-(tetrachlorobis(1H-indazole)ruthenate(III)), which is currently in Phase I/II trial, is already known for its promising anticancer activity. In course of a recent screening using diverse cancer cell lines of different origin (n=22), a small subgroup with exceptional sensitivity to KP1339-treatment was discovered. The aim of the here presented study was to identify factors that underlie this sensitivity or which are connected to resistance to KP1339. Subsequent investigations confirmed that upon KP1339-treatment hypersensitive cell lines showed pronounced apoptosis induction (indicated by caspase-mediated PARP cleavage and appearance of apoptotic nuclei visualized by DAPI staining). In contrast, normal responsive cells rather reacted with prolonged duration of the G2 phase to KP1339 treatment (video evaluation). This was confirmed by FACS analysis of ethanol-fixed and propidium iodide-stained cells indicating a profound increase in the percentage of cells with double DNA content upon KP1339 treatment. Moreover, in accordance to previous studies, KP1339 induced strong phosphorylation of ERK, p38 and JNK especially in normal responsive cell lines. This is of
interest as p38 and JNK activation are known to be pro-survival factors within the extrinsic pathway of apoptosis. The relevance of this particular pathway is supported by results of activation of caspase 8 upon KP1339 treatment in hypersensitive cells (Western Blot). Verification of functional importance of caspase 8 activation was attained through combination assay with KP1339 and caspase 8-inhibitor Z-IETD-fmk. In this assays, Z-IETD-fmk was able to reduce apoptosis levels in KP1339-treated Capan1. This finding will help to better understand the mechanisms underlying the promising anticancer activity of KP1339.

176 The role of STAT5A and STAT5B in leukemia


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Expression of the BCR-ABL oncogene is associated with chronic myeloid leukemia (CML) and B cell acute lymphoblastic leukemia (B-ALL). BCR-ABL orchestrates a network of downstream signaling molecules in which the transcription factor STAT5 represents a key point of intersection crucial for survival and proliferation of leukemic cells. There are two known isoforms of STAT5, designated STAT5A and STAT5B, with approximately 90% sequence homology. Recently, somatic gain-of-function mutations of STAT5B were identified in large granular lymphocytic leukemia, T cell acute lymphoblastic leukemia, T cell prolymphocytic leukemia, γδ hepatosplenic T cell lymphoma, and NK cell lymphoma, while mutations of STAT5A were not reported in these diseases so far. To identify differences in growth characteristics between STAT5A and STAT5B, we overexpressed either protein in murine and human CML and B-ALL cell lines. Our preliminary in vitro experiments suggest that overexpression of STAT5A is advantageous for cell proliferation of CML cell lines, while overexpression of STAT5B conferred a growth advantage only to B-ALL cell lines. We hypothesize that STAT5A and STAT5B 1) are differentially regulated in their expression, 2) interact with different epigenetic cofactors, and / or 3) differentially regulate the expression of target genes. We aim to identify these differences via chromatin immunoprecipitation (ChIP), ChIP-seq, rapid immunoprecipitation mass spectrometry of endogenous proteins (RIME), and RNA-seq. This work is funded by the FWF grant P 24295 and the FWF-funded doctoral program W 1212 Inflammation and Immunity.

177 Role of the AP-1 protein c-Jun in plasmacytoid dendritic cells

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Cancer is one of the leading causes of death in the industrialized world. Every third diagnosed cancer is a skin cancer. Imiquimod (Imi) is an immune modifying compound used as a 5% cream formulation (Aldara) to treat warts and basal cell carcinomas (BCC). The mechanism of action of Imi relies on the activation of Toll like receptor 7/8 (TLR7/8) expressing immune cells, prominently a subtype of dendritic
cells called plasmacytoid dendritic cells (pDCs). pDCs are Type I interferon producing innate immune cells. We have recently shown that if activated they can be converted into tumor killing cells. The tumor killing ability of pDCs is independent of adaptive immunity and relies on the production of lytic molecules like Granzyme B (Gzmb) and Tumor necrosis factor related apoptosis inducing ligand (TRAIL). The production of these tumor killing molecules in pDCs as well as other pro-inflammatory molecules like tumor necrosis factor alpha (TNF-α) or Type I Interferon are controlled by a defined subset of transcription factors like interferon regulator factor 7 (IRF 7) and nuclear factor 'kappa-light-chain-enhancer' of activated B-cells (NFκB). Another well known family of immune regulators is the AP-1 family whose role in pDCs and Imi mediated tumor clearance is poorly understood. In order to investigate the role of c-Jun in pDC development and function, we are employing mice harbouring floxed c-Jun alleles to delete c-Jun in all bone marrow (BM)-derived cells with the poly I:C inducible Mx-Cre transgenic line or in Dendritic Cells only by using the CD11c-Cre line. Our results indicate that c-Jun is dispensable for the development and maturation of pDCs. Furthermore, we could show that c-Jun is an important factor for the production of Interleukin-6 (IL-6) and Interferon beta (IFN-β) in Imi stimulated pDCs. Current studies are addressing the tumor-killing capacity of Imi-stimulated pDCs.

178 Oncogenic Role of STAT1 in intestinal tumorigenesis

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179 Investigating the NAD metabolome in Ewing Sarcoma cells

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Ewing Sarcoma (ES) is the second most common bone cancer in children and adolescents with a high metastatic potential. Tumor development is driven by the specific t(11;22)(q24;q12) chromosomal translocation resulting in generation of the chimeric transcription factor EWS-FLI1. Recently, ES has been reported to be exquisitely sensitive to inhibitors of poly(ADP-ribose) polymerase 1 (PARP1). This enzyme uses NAD as substrate and was demonstrated to regulate EWS-FLI1 in a feed-back mechanism. NAD plays a central role in cellular redox reactions, DNA repair, and in the maintenance of genomic stability. Usually, NAD is regenerated from nicotinamide in the NAMPT-dependent salvage pathway or from the reduction of pyruvate via LDHA (Warburg effect), but can also be synthesized de novo from tryptophan.
Interestingly, the knockdown of EWS-FLI1 in ES cells comes along with alterations in the expression of multiple enzymes involved in NAD biosynthesis and regeneration. We are interrogating the role of EWS-FLI1 mediated modulation of these enzymes and of specific small molecule inhibitors on cellular tryptophan consumption, kynurenine production and intracellular NAD levels of Ewing sarcoma cells. Preliminary results suggest that NAMPT inhibition diminishes PARP1 activity due to low NAD, implying a pivotal role for the regenerative salvage pathway in ES cells. These studies aim at a better understanding of factors influencing Ewing sarcoma sensitivity to therapies targeting PARP1 and the NAD metabolome.

MRTF and EWS-FLI1 define the Ewing Sarcoma transcriptional network

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The pediatric bone cancer Ewing sarcoma (ES) is shaped by the expression of the chimeric transcription factor EWS-FLI1, derived from a chromosomal translocation. The clinical challenge in ES treatment is the early onset of metastasis. Metastasis is a complex process, which requires a high degree of cellular plasticity. Recent studies show that EWS-FLI1 deregulates the actin cytoskeleton, which is furthermore regulated predominantly by the Rho pathway. Upon activation it promotes the polymerization of G-actin into stress fibers (F-actin) thereby releasing the myocardin-related transcription factors (MRTF-A, MRTF-B). MRTFs translocate to the nucleus and bind to the global transcription factor serum response factor (SRF). However, the ternary complex factors (TCF), activated downstream of Ras, competes with MRTFs for SRF binding in the presence of ETS binding sites adjacent to the SRF consensus sequence. The ETS-transcription factor EWS-FLI1 has been previously shown to form a complex with SRF. We hypothesize that EWS-FLI1 competes with MRTFs for SRF binding and uncouples transcriptional regulation from Rho. Upon combinatorial knockdown of MRTFA/B and EWS-FLI1 in two ES cell lines we demonstrate that in the absence of the fusion oncogene, MRTF can inversely regulate the majority of EWS-FLI1 target genes. These results are currently validated by ChIP sequencing. Interestingly, we found that MRTF transcriptional effects are already present in the absence of serum in ES cells concordant with constitutive nuclear MRTFA localization. A small panel of MRTF target genes, however, regains serum inducibility upon EWS-FLI1 knockdown. By using an actin-polymerization inhibitor, LatrunculinB, we demonstrate that MRTFB but not MRTFA is responsive to serum induced actin assembly, indicating that MRTFA is uncoupled from this regulation. These findings reveal a previously unknown role of MRTF in EWS-FLI1 driven transcriptional dysregulation.

The role of Epidermal Growth Factor Receptor in c-Fos-dependent osteosarcoma formation

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Melanoma is the most aggressive type of skin cancer and its incidence rises worldwide. Metastatic melanoma displays a three years overall survival rate of less than 15%. So far no effective treatment is available and most patients acquire resistance during therapy. Disturbed homeostasis of the endoplasmic reticulum (ER) - termed ER stress - is found in various cancer types and is considered as double-edged sword in tumor progression. On the one hand, it enables adaption to altered metabolic demands; on the other hand it triggers apoptosis. In this project we present data indicating that ER stress triggers melanoma malignancy. Using isogenic cell lines we show that ER stress is increased in metastatic compared to non-metastatic melanoma cells. The ER stress branches ATF6 and PERK - but not IRE1 - are selectively activated in metastatic cells. Meta-analyses of available data from the literature confirmed that ATF6 and PERK activation is associated with poor survival in human melanoma patients. Furthermore, ER stress was detected in metastatic melanoma cells residing in lymph nodes in a melanoma mouse model. Microarray and gene set enrichment analysis revealed that ER stress in melanoma cells up-regulates the expression of genes involved in migration, invasion and angiogenesis. Indeed, amelioration of ER stress using the chemical chaperon 4-PBA decreased invasion and migration of metastatic melanoma cells in-vitro. Taken together, we show that ER stress promotes melanoma malignancy by enhancing migration and invasion. Thus, ER stress has to be considered a potential therapeutic target in metastatic melanoma.
Melanoma is a highly invasive and metastatic type of cancer with poor prognosis. In melanoma BRAF is the most frequent oncogene that induces the constitutive activation of the RAS-RAF-MEK-ERK signaling cascade. While Ca2+ signaling is a well-known regulator of tumor progression, the crosstalk between Ca2+ signaling and the MAPK pathway is much less explored. Plasma membrane calcium ATPases (ATP2B/PMCA) maintain the resting low intracellular calcium concentration by pumping out excess calcium from the cytosol. Changes in PMCA expression during malignant transformation have been described in several tumor types but not in melanoma so far. Here we show that in BRAF mutant melanoma cells the protein level of the plasma membrane Ca2+ATPase isoform 4b (PMCA4b) is markedly elevated by the treatment of mutant BRAF specific inhibitor vemurafenib. Furthermore, we demonstrate that the MEK inhibitor selumetinib increases PMCA4b abundance levels in both BRAF and NRAS mutant melanoma cells suggesting that BRAF/MEK signaling has an important role in the regulation of PMCA4b abundance. In accordance with the increased abundance of PMCA in the plasma membrane, an
enhanced [Ca2+]i clearance is observed in the vemurafenib-treated BRAF mutant cells. Both vemurafenib treatment and PMCA4b overexpression inhibited the migration of BRAF mutant melanoma cells. However, while vemurafenib inhibits proliferation of the cells through the inhibition of ERK activation, PMCA4b overexpression itself did not influence it. Furthermore, we show that the reduced migration of the PMCA4b expressing BRAF mutant cells is associated with a marked decrease in their metastatic potential in vivo. Taken together, our data reveal an important crosstalk between Ca2+ signaling and MAPK pathways through the regulation of PMCA4b expression and suggest that PMCA4b is a previously unrecognized metastasis suppressor. Supported by the Hungarian Scientific Research Funds K101064 and ANN110922; and API01662FW.

185 The Role of Plasmacytoid Dendritic Cells in Imiquimod Induced Skin Inflammation and Melanoma Clearance in Mice

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186 Serum-dependent processing of late apoptotic cells for enhanced efferocytosis

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Binding of complement component C1q to the surface of dying cells facilitates their clearance by phagocytes in a process termed efferocytosis. Here we investigate during which phase of the apoptotic cell death progression the C1q binding takes place and whether this is influenced by other serum factors. Four different chemotherapeutic drugs (oxaliplatin, irinotecan, etoposide, and 5-FU) and UV-C irradiation were applied to induce apoptosis in human leukemic Jurkat T-cells. Apoptosis was confirmed by Western blotting and cell volume measurements. C1q binding and phagocytosis was assessed by flow cytometry. Incubating apoptotic cells in normal human serum [NHS] resulted in the formation of a subpopulation of late apoptotic / secondary necrotic cells which showed a specific strong binding of C1q. This occurred independently of the apoptosis inducer and could also be observed in other cell types. C1q-binding cells exhibited a smaller volume, a more degraded protein composition, and a lower DNA content in comparison to the remaining late apoptotic / secondary necrotic cells and were therefore considered as apoptotic bodies. In contrast, purified C1q was found to bind to all dying cells and, albeit weaker, also to viable cells. In addition, apoptotic cells incubated with purified C1q showed no DNA degradation. Co-culturing NHS-treated cells with human monocytes revealed a much higher phagocytosis of C1q-positive than of C1q-negative late apoptotic / secondary necrotic cells. However, this phagocytosis-promoting activity could not be observed with purified C1q. These results show that serum factors are involved in the prevention of C1q binding to viable cells and in the processing of late apoptotic / secondary necrotic
cells promoting cell death progression towards apoptotic bodies. The latter process leads to the exposure of new C1q binding structures which facilitates efferocytosis.

187 CHARACTERIZATION OF SOMATIC GAIN- OR LOSS-OF-FUNCTION MUTATIONS OF STAT5 IN CANCER


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STAT5A/B transcription factors are part of the JAK-STAT core cancer pathway, and function as critical downstream mediators of cytokine and growth factor activity. STAT5 proteins form oligomers and dimers through their N-terminal domain and STAT5 oligomers have been suggested to support the process of chromatin reconfiguration thus, regulating the transcription of target genes. Furthermore, STAT5 proteins have multiple phosphorylation, methylation and acetylation sites at their C-terminal domain. Tyrosine phosphorylation of STAT5 promotes SH2 domain mediated parallel dimerization and nuclear translocation. Through human cancer genome sequencing efforts, it has become clear that recurrent hot spot mutations cluster predominantly in STAT5B, driving T cell leukaemia and defining it as a new drug target. Currently, there are 86 mutations found in STAT5B with more than 40% of which being located on the SH2 and the C-terminal domain of these proteins, provoking particular interest. Some of these point mutations are potential gain-of-function (GOF) or loss-of-function (LOF) mutations, however, their biochemical characteristics to date are poorly understood. We aim to examine the biochemical behaviour of these somatic mutations together with their affects on STAT5 activation and protein-protein interactions. GOF and LOF mutations of STAT5 are studied based on their ability to induce interleukin-3-independent growth of Ba/F3 cells. Their DNA binding capability, dimers and oligomer formation potential as well as their transcriptional activity is analyzed in detail. Finally, the oncogenic potential of the identified GOF or LOF variants are examined in transgenic murine models or studied using transplantation experiments.

188 Light-induced activation of mesothelioma cell growth and malignant behavior via Opto-FGFR

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Fibroblast growth factors (FGFs) and their receptors (FGFRs) comprise a potent intracellular signaling system regulating cell growth, survival and aggressiveness. Malignant Pleural Mesothelioma (MPM) is a tumor characterized by different histological subtypes: epithelioid and sarcomatoid MPM show flat and spindle-shaped cell morphologies, respectively, whereas biphasic MPM contains a mixture of both. Patients suffering from non-epithelioid MPM have a worse prognosis. Previous data showed that FGF2 induces MPM cell growth as well as morphological changes reflecting epithelioid to sarcomatoid appearance in two biphasic cell lines. We engineered an Opto-FGFR1, a FGFR1 which lacks the ligand binding domain and therefore is insensitive to endogenous ligands, but instead can be activated by blue light enabling contactless spatially and temporally precise activation. This was possible by linking a light-oxygen-voltage (LOV)-sensing domain from a phototrophic organism to the catalytic domain of the receptor. Opto-FGFR1 was stably expressed in MPM cells via retroviral particles. Cells were then stimulated with light for different times. Activation of the receptor and downstream signals was assessed by western blot and immunocytochemistry. Cell proliferation was measured by BrdU-incorporation and changes in cell morphology were characterized using microscopic images. Stimulation of the transgenic cells with light resulted in increased cell growth as well as activation of FGFR1 and the MAPK pathway. Similar to FGF2 treatment, changes from an epithelioid into a sarcomatoid morphology in two biphasic MPM lines were observed which could be prevented by pharmacologic FGFR1 inhibition. In summary, light-activated Opto-FGFR1 was able to mimic complex morphogenic and mitogenic tumor cell behavior induced by FGF2. Light activation of Opto-FGFR1 with temporal and spatial precision provides a powerful new tool to study and manipulate cellular signals and behavior.

189  **In vitro activation of tumor-infiltrating lymphocytes**

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Immune cells were found to play a major role in the progression of colorectal cancer. Tumor-infiltrating lymphocytes were shown to be the strongest prognostic factor of the disease. There is evidence that the distribution of different immune cell subtypes mirrors the anti-tumor immune response in the patient. However, the classification of these immune cells is still insufficient, especially for tumor-associated macrophages. Thus, we intend to classify tumor-infiltrating lymphocytes and macrophages by using mass spectrometry and live cell preparation. The latter approach focuses on the isolation of immune cells from the tumor tissue and stimulation of these cells. We succeeded in isolating CD3+ cells (T cells) and CD19+ cells (B cells) from colon tumor tissue. In addition, in vitro stimulation of the isolated cells revealed that these cells are able to produce INFγ. In a first step using mass spectrometry we can show that CD14+ cells (monocytes) isolated from human blood differ from the remaining CD14- cells in their protein expression profile. The next step is to identify specific lymphocyte- and macrophage-spectra in tumor
sections. In conclusion, we can analyze various cell types by mass spectrometry, and isolate as well as stimulate lymphocytes isolated from tumor tissue.

190 **In vitro cytotoxic activities of the oral platinum(IV) prodrug oxoplatin and HSP90 inhibitor ganetespib against a panel of gastric cancer cell lines**

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Gastric cancer exhibits a poor prognosis and is the second most common cause of cancer death worldwide. Chemotherapy of metastatic gastric cancer is based on combinations of platinum drugs and fluoropyrimidines, with added agents. Oxoplatin is a stable oral platinum(IV) prodrug which is converted to a highly active tetrachlorido(IV) complex under acidic conditions. In the present work we studied the cytotoxic effects of oxoplatin against a panel of four gastric cancer cell lines in vitro. Additionally, the role of HSP90 in chemoresistance was investigated using the specific inhibitor ganetespib. The KATOIII, MKN1, MKN28 and MKN45 cell lines were used in MTT, cell cycle, Western Blot and apoptosis assessment tests. Markers of apoptotic cell death/stress were investigated with proteome profiler arrays and M30/M65 cytokeratin fragments ELISA kits. Interactions of platinum drugs and ganetespib were calculated with the Chou-Talalay method. Oxoplatin revealed low activity against the used cancer cell lines, whereas the platinum tetrachlorido(IV) complex and cisplatin gave IC50 values of 1-3µg/ml with increasing chemoresistance observed in the order of MKN1, KATOIII, MKN28 to MKN45. Release of the M30 caspase-cleaved cytokeratin fragment and increased expression of cleaved caspase3 and claspin in response to drug treatment are markers of apoptosis and cell cycle arrest, respectively. With exception of KATOIII and MKN28/oxoplatin, all other cell lines featured marked synergistic toxicity with clinically achievable concentrations of ganetespib. Furthermore, HSP90 seems to play an important role in the proper folding and expression of the pH regulator carbonic anhydrase IX. Oxoplatin seems to constitute an oral platinum prodrug whose metabolite exhibited activity comparable to cisplatin. Oral administration of a platinum agent would be of great value for patients. The results suggest that HSP90 represents an important mediator of chemoresistance in gastric cancer.

191 **Qualitative and quantitative analyses of B-cell subsets in follicular structures: from classical germinal centers to ectopic follicles at tumor site**

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B-cell-attributed imprint of the tumor microenvironment has been shown to influence disease outcome in some types of cancers. Recent study of our group demonstrated that accumulation of CD20+ B cells at the metastatic site of patients with colorectal cancer metastases in the liver and/or their assembling into ectopic follicles are strongly associated with a better prognosis. Yet, the biological mechanisms for development and maintenance of germinal center (GC)-like ectopic structures within malignant tissue are not well defined; limited knowledge is available on the magnitudes of post-germinal memory and/or plasma cell subsets. Herein we develop a microscopy-based strategy allowing quantitative assessment of memory and plasma B cells across large-scale tissue specimens. We first assessed the B-cell differentiation pattern in classical follicles within tonsil tissue. We used CD20, AID, IgM, CD27, CD73, and CD138 as B-cell subset markers. Given the broader expression pattern of CD27 and CD73, we discriminated the IgM+/CD27+, IgM+/CD73+ or CD20+/CD27+ memory cells and CD138+/CD27high plasma cells. Upon staining we applied sophisticated analysis using the microscopy-based TissueFAXS platform assessment of various populations within predefined compartments. Thereby lymphoid follicles were subdivided into the following compartments: GC, mantle zone and surrounding which allows qualitative and quantitative rim of 100µm. The results show the exact distribution of various B-cell subsets across the follicular compartments and indicate the follicle- and specimen-specific differences in the balance between memory and plasma cells. Within ongoing study the established strategy is applied to characterize the ectopic follicles formed within primary colon cancer and at the metastatic site in the liver. This is the first step to highlight the potential immunomodulatory mechanisms underlying the antitumoral effects of local B-cell responses. Supported by FWF P22441-B13/P23228-B19

Small cell lung cancer (SCLC) is characterized by a high aggressiveness and an early progression to metastasis. Activins, cytokines that have an important role in inflammation, growth, cell death and wound healing, are members of the TGF-β family of growth and differentiation factors. Activin A, the dimer of two beta A subunits has been described to impact on malignant growth in different types of solid tumors. We have observed increased levels of activin A in the circulation of subsets of SCLC patients. To investigate the impact of activin A on the malignant behaviour of SCLC cells, transgenic cell lines overexpressing activin A were generated by using retroviral transduction. For characterizing
growth, viability, cell proliferation and cell cycle distribution upon an elevated level of activin A, growth, MTT, clonogenic assays as well as flow cytometry of propidium iodide stained cells were performed. An in vitro co-culture system using endothelial cell monolayers and tumor cell spheroids was used to investigate bulk invasion of tumor cells into lymph and blood vessels (gap formation assay). Anchorage independent growth was measured by performing a soft agar colony formation assay. Results revealed no significant difference in growth, viability or cell cycle distribution between activin A overexpressing SCLC cells and the respective control group. There was also no significant difference in gap forming activity in blood endothelial cell monolayers. In lymphatic endothelial cells, however, SCLC spheroids with an increased level of activin A generated a significant higher level of circular defects. In the anchorage independent growth assay, activin A overexpressing cells also demonstrated a greater capacity to form colonies. The results show that in SCLC cells an elevated level of activin A has a no effect on cell proliferation but increases properties relevant for metastasis such as lymphendothelial gap formation and colony forming ability in soft agar.

193 Regulation of the plasma membrane Ca\textsuperscript{2+} pump PMCA4b by mutated BRAF

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The BRAF mutation V600E occurs in about 8% of human tumors with an oncogenic influence on cell survival and proliferation in melanoma. The mutant BRAF-specific inhibitor vemurafenib blocks the constitutive active MAPKinase pathway, resulting in positive therapeutic effects in melanoma. Nevertheless development of resistance and limited response require discovery of additional molecular targets. Aberrant plasma membrane Ca\textsuperscript{2+} ATPase (PMCA4b) expression and dysregulation of Ca\textsuperscript{2+} homeostasis appears in various malignant cancers. PMCA proteins are important regulators of intracellular Ca\textsuperscript{2+} homeostasis with cell type specific distribution. Increased Ca\textsuperscript{2+} influx promotes migratory ability and metastatic activity in melanoma and suggests a possible crosstalk between BRAF mutation and PMCA4b aberration. MAPKinase pathway inhibition in BRAF mutant melanoma with
vemurafenib markedly increased the PMCA4b protein abundance. In order to investigate the regulation of PMCA4b expression on the transcript level, quantitative real time PCR assays were performed and showed only a modest PMCA4b expression increase in one of two BRAF mutant melanoma cell lines, indicating a mainly posttranscriptional regulation. Additionally, epigenetic effects of HDAC inhibitors on PMCA4b expression are analyzed alone and with vemurafenib combined treatment. To investigate the hypothesis that mutant BRAF represses PMCA4b expression, wild type and V600E mutant BRAF were cloned into retroviral expression vectors. Colon cancer cells, transduced with these BRAF retroviruses are used to further test the crosstalk between mutant BRAF activity and PMCA4b expression and activity. The summarized data provide evidence for a suggested crosstalk between MAPKinase pathway inhibition and PMCA4b-mediated Ca\(^{2+}\) signaling regulation, further investigated in melanoma and colon cancer. Supported by the Hungarian Scientific Research Funds K101064 and ANN110922; and the Austrian Science Fund API01662FW.

194 NK cell biology - another leading role for CDK6?

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CDK6 and its close homolog CDK4 are important cell-cycle kinases that regulate the transition from the G1 to the S-phase of the cell cycle. CDK6 was found to be overexpressed in several hematological malignancies and CDK4/6 inhibitors are already FDA approved for breast cancer patients. Recently, we identified CDK6 as a key player in leukemogenesis as well as in normal and leukemic stem cells. Additionally, we could demonstrate that CDK6, but not CDK4, acts as a transcriptional regulator in a kinase-independent manner. Natural killer (NK) cells comprise about 15% of circulating lymphocytes, are key players of the innate immune system and provide immediate defense against pathogens and cancer cells. To test the impact of CDK6 on NK cells we analyzed the development, the maturation, the receptor repertoire and the cytotoxic properties of NK cells deficient for CDK6. We could show that loss of CDK6 alters NK cell development and maturation. Furthermore, NK cells deficient for CDK6 display accelerated NKG2A/C/E and NKG2D receptor expression, suggesting that CDK6 has an impact on NK cell activation. These results led us to speculate that loss of CDK6 might have consequences for cytotoxic functions and NK cell mediated tumor cell clearance. To confirm this, we plan to perform in vitro cytotoxicity assays and challenge CDK6-deficient mice with B16F10 melanoma cells. To determine whether kinase-dependent or -independent functions of CDK6 are accountable for NK cells we will also characterize NK cells of kinase-dead CDK6-K43M mutant mice. These experiments are of utmost importance to understand and predict potential consequences of CDK6 inhibitors on NK cell development and function. This work is supported by the DK Inflammation and Immunity and the FWF grant #F24297-B23.

195 Genotoxicity of TiO\(_2\) -based carrier for metallo-drugs and its influence on the antioxidative cell status

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Transition metal complexes have been used in anti-tumor therapy more than five decades. Although they are efficient in tumor killing, serious side effects and resistance limit their use. In general, transition metal complexes suffer of low solubility in physiological solutions, which might cause a problem with the concentration that reaches the target cell. These drawbacks can be overcome either by synthesis of new generations of metallo-drugs or by developments of drug carrier. TiO2 as a material for drug carrier has attracted a lot attention due to its properties, such as stability, availability, biocompatibility and possibility for surface modification. In this work, we are focused to test the possibility of use colloidal TiO2 nanoparticles (NPs) as a carrier for transition metal complex-potential anti-tumor agent. For that purpose, we have synthesized Ru-complex (cis-dichlorobis(2,2'-bipyridyl-4,4'-dicarboxylic acid)ruthenium(II)), with ligand suitable for interaction with the surface of TiO2 NPs. Complex-TiO2 nanosystem was formed, and together with nanosystems´ components tested for genotoxicity as well as its effect on the antioxidative status of peripheral blood cells. Results show that both Ru-complex and colloidal TiO2 NPs increase the frequency of micronuclei and inhibit cell proliferation. On the other hand, nanosystem seems to have less pronounced effects. Further, our results shows that only TiO2 NPs affected the activity of catalase by inhibition while nanosystem and nanosystems´ components induces the content of malondialdehyde, as the measure of free radical production in the cells. Studies with cellular systems imply that all component of the synthesized carrier system induce cellular damage, either via influence on the genetic material or through the disturbance of the oxidative balance and damage of phospholipids, which make them potentially interesting from the standpoint of development of more efficient anti-tumor therapy.

196 Expression of Thyroid hormone binding protein µ-crystallin (CRYM) in the development and progression of prostate cancer and its role in the invasion, migration and apoptosis

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Prostate cancer is the most common cancer in men worldwide and millions of men are diagnosed with this disease each year. Androgens play an important role in the early stages of prostate cancer and androgen receptor (AR) signalling has been shown to be involved in differentiation and apoptosis of prostate cancer cells, but the underlying molecular mechanisms are incompletely understood. In this study, we investigated the role of the thyroid hormone binding protein 1/4-crystallin (CRYM) and its relation to androgen receptor signaling and thyroid hormone regulation in the development and progression of prostate cancer. We evaluated CRYM expression levels in a panel of prostate cancer cell lines and human patient samples. CRYM expression was detected in androgen-dependent LnCAP and RPWE-1, but not in other cells such as VCAP, PC3, DU145, LAPC-4 AND 22RV-1. CRYM is highly expressed in normal prostate tissues but it is continuously decreased in primary, metastasis and therapy resistant patients. Intracellular T3 hormone-binding capacity is increased in cells with high expression of cytosolic thyroid hormone-binding protein μ-crystallin (CRYM) and overexpression of CRYM decreased the invasion of PC3 and DU145. Furthermore, CRYM expression is correlated with the poor prognosis of patients by analysing the biochemical recurrence of PSA in prostate cancer. In summary, the identification of CRYM as a thyroid hormone binding protein might be of prognostic but also therapeutic relevance in patients with prostate cancer.

197 Targeting STAT5 in myeloproliferative neoplasms

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STAT5 proteins are key transcription factors that gain increasing attention as essential drivers in the development of myeloproliferative diseases. STAT5 is often found to be hyper-activated due to upstream disruptions of the kinase signaling pathway. However, point mutations in conserved domains of STAT5 itself were described, implying its importance in oncogenic transformation independent of driver kinase mutations. Considering the role which STAT5 proteins play in various MPNs, it is reasonable to target STAT5 as transcriptional regulator instead of using small molecule inhibitors of upstream activators since such inhibitors suffer from the development of resistance, cytotoxicity as a result of poor kinase selectivity, as well as cardiovascular toxicity. The major aim of this study is to specifically inhibit activation and DNA binding of STAT5 to block activation, survival and proliferation of neoplastic cells and improve existing therapies in MPNs. To inhibit STAT5, we use a small inhibitory molecule binding to the SH2 domain of the protein, resulting in the disruption of STAT5-phosphopeptide interactions. In MPN cell lines, the inhibitor can suppress STAT5 phosphorylation and STAT5-mediated gene expression as shown by Western Blot analysis. Importantly, we detect down regulation of c-MYC, Cyclin D1, Cyclin D2, and MCL-1 oncoproteins, which are important inhibitors of apoptosis and regulators of the cell cycle. We can also show a significant increase in apoptosis via AnnexinV/PI staining and cell cycle inhibition via PI
staining. Furthermore, STAT5 DNA binding ability is efficiently blocked as analyzed with EMSA assay. Our findings indicate a potential of the STAT5 inhibitor as an anti-cancer compound. However, further investigations have to be done to validate the results from the experiments with human MPN model cell lines. Thus, we aim to analyze the effect of the compound on MPN patient cell lines and mouse models.

**198 The TYK2-pathway as a novel therapeutic target in aggressive T-Cell Lymphoma**

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Anaplastic lymphoma kinase (ALK) inhibition has shown remarkable success in targeted therapy of non small cell lung cancer and in anaplastic large cell lymphoma (ALCL) where ALK kinase fusion proteins have initially been identified (ALK+) in 60% of the patients. ALCL is a CD30 positive, aggressive Non-Hodgkin T-cell lymphoma mainly affecting children or young adults. Unfortunately treatment with ALK inhibitors inevitably leads to resistance development making the identification of new therapeutic targets an imperative. To achieve this we screened primary T-cell lymphoma samples and ALCL cell lines with a preselected library of 105 substances with known molecular targets and found that cells were highly susceptible to inhibitors of tyrosine kinase 2 (TYK2), a member of the Janus kinase family (JAK1, JAK2, JAK3, TYK2). In contrast, peripheral blood mononuclear cells (PBMC) and the JURKAT T-ALL cell line were not affected. In order to corroborate the importance of TYK2 for ALCL cell survival shRNA mediated gene knockdown was performed resulting in strong induction of apoptotic cell death. Analysis of potential downstream targets (STAT1, STAT3, STAT5) revealed distinct p-STAT1 reduction after small molecule TYK2 inhibition. Accordingly, shRNA mediated gene knockdown of STAT1 revealed an essential function for ALCL cell survival. Taken together, our results indicate TYK2-pathway dependence in ALCL. Thus we identified a novel therapeutic intervention site that is druggable by recently developed small molecule inhibitors. Ongoing in vivo experiments using an ALCL transgenic mouse model in which TYK2 is knocked out in T-cells will systematically assess the therapeutic relevance of our findings.
Critical common effectors of the oncogenic NUP98 multi-partner translocation family in acute myeloid leukemia

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Acute myeloid leukemia (AML) is the most frequent form of myeloid blood cancer and is often associated with poor prognosis. Many disease-defining entities in AML represent specific translocation-associated fusion genes that are clustered in multi-partner translocation (MPT) families. In these MPT families one conserved partner moiety is fused to various different recipient loci, thereby creating related fusion proteins. With more than 20 known fusion partners, translocations involving the Nucleoporin 98 (NUP98) gene are among the largest MPT families. Many NUP98-fusion proteins can act as strong driver oncogenes, but it is unknown if NUP98-MPT fusions share conserved mechanisms of leukemic transformation. Up to now, only isolated studies on the oncogenic mechanism of certain NUP98 family members have been performed. We hypothesize that distinct NUP98 fusion proteins share common oncogenic mechanisms that are crucial for the development and maintenance of AML and that these mechanisms can be identified using a systematic, comparative approach. Five relevant in vivo models of different NUP98-fusion-protein-driven leukemia were established and will be used to analyze networks of physical, genetic and epigenetic interactions of the selected NUP98 fusions with key effector proteins. Affinity purification coupled to mass spectrometry (AP-MS) of tagged fusion proteins from ex vivo-isolated leukemic blasts will be used to identify common protein interactors. RNA sequencing will be employed for gene expression profiling. The functional contribution of selected candidate genes to leukemogenesis will be tested by pooled shRNA screening and high-confidence hits will be further validated through detailed loss-of-function and gain-of-function studies. Results from this project may fundamentally improve our understanding of the molecular pathways that are engaged in NUP98-driven leukemia and might reveal vulnerable targets for the development of novel therapeutic compounds.

Mitochondrial signaling and p53 as key determinants of KP46 anticancer activity

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The neoplastic gallium compound, tris(8quinolinolato)galliumIII, KP46, was recently developed at the Institute of Inorganic Chemistry at the University of Vienna. KP46 was successfully evaluated in phase I clinical trials and is currently heading for phase II evaluation. However, the exact molecular cytotoxic mode of action in tumour cells is still not well understood.

Using HCT116 colon cancer cells, carrying functional or deleted p53, the effects of KP46 on mitochondrial mediated apoptosis and autophagy were studied in detail. In addition to protein and gene expression analyses, fluorescence microscopy and flow cytometry, real-time monitoring of cell viability utilizing the xCELLigence system, oxygen consumption and extracellular acidification using the Seahorse Biosciences analyser and spectrometric measurements assessing the intracellular cations and the mitochondrial permeability transition were carried out.

We found that KP46 accumulated in mitochondria, triggering structural and functional mitochondrial damage and furthermore drastically lowered the intracellular labile iron pools. Consequently, p53 accumulated in the nucleus and activated its downstream target Bnip3L, a protein with essential functions in apoptosis and mitophagy. KP46 triggered Bnip3L dependent PARKIN mediated mitophagy and sensitized the opening of the mitochondrial permeability transition pore. Our data indicates that targeting Bnip3L in the presence of p53 is a novel anticancer strategy including iron depletion and mitophagic signaling as cell death inducers.

201 Cooperation of ETV6/RUNX1 and BCL2 leads to accelerated disease progression in a transgenic mouse model

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The (12;21) chromosomal translocation resulting in the ETV6/RUNX1 (E/R) fusion is the most frequent translocation in childhood B cell precursor acute lymphoblastic leukemia. The fusion is detectable already in utero but the outbreak of the disease occurs after years, with the highest incidence in children between 3 and 6 years, suggesting that the E/R fusion alone is not sufficient for leukemia development. Aberrant expression of BCL2 family genes was recently observed in the context of E/R-positive leukemia. In this study, we focused on anti-apoptotic BCL2 and investigated cooperative effects of E/R and BCL2 in a transgenic mouse model.

We crossed mice expressing the E/R fusion with mice that overexpress BCL2. Double transgenic (dtg) mice died significantly earlier when compared to their diseasing BCL2tg counterparts. Both groups developed lymphomas. Interestingly, dtg mice are more affected at younger age, while in older mice the phenotype is more pronounced in BCL2tg mice. Kidney histology revealed an immunocomplex glomerulonephritis (GN). The significantly higher intensity of IgG deposits in glomeruli of dtg mice led us to the hypothesis that E/R could drive immunoglobulin (Ig) production. Serum ELISAs of diseased mice confirmed a trend for higher Ig levels in dtg mice. Further, we found GN in all examined dtg mice while in BCL2(tg) mice some older animals had only a very weak GN phenotype. We speculate that dtg mice die as a consequence of the GN while BCL2(tg) mice can overcome or do not develop GN in some cases which leads to a stronger phenotype in other organs.
We hypothesize that elevated numbers of B cells due to the expression of E/R in concert with anti-apoptotic BCL2 lead to more immunoglobulin production, and finally to the deposition of immune complexes in glomeruli and the faster development of immunocomplex GN in dtg mice.

**202** EGFR is required in liver macrophages for IL-1-induced IL-6 production and hepatocellular carcinoma formation


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Hepatocellular carcinoma (HCC) is the sixth most frequent cancer with limited treatment options and poor prognosis. Tumorigenesis has been linked with macrophage-mediated chronic inflammation and diverse signaling pathways including the Epidermal Growth Factor Receptor (EGFR) pathway. The precise role of EGFR in HCC is unknown, and EGFR inhibitors have shown disappointing results in clinical trials likely due to the lack of biomarkers allowing patient stratification. Here we discover that EGFR is expressed in liver macrophages in both human HCC and in a mouse HCC model. Mice lacking EGFR in macrophages show impaired hepatocarcinogenesis, whereas mice lacking EGFR in hepatocytes unexpectedly develop more HCC due to increased hepatocyte damage and compensatory proliferation. The presence of EGFR-positive liver macrophages in HCC patients is associated with poor survival. Mechanistically, following IL-1 stimulation, EGFR is required in liver macrophages to transcriptionally induce IL-6, which triggers hepatocyte proliferation and HCC. This study highlights the complexity of EGFR signaling in HCC and demonstrates a new tumor-promoting mechanism for EGFR in non-tumor cells, which could lead to more effective precision medicine strategies.

**203** Infrared A Radiation Promotes Survival of Human Melanocytes Carrying UVR-induced DNA Damage


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While it is widely accepted that Ultraviolet Radiation [UVR] is a main risk factor for non-melanoma skin cancer, the link between solar radiation and melanoma is still a matter of debate. Although Infrared Radiation (IR) accounts for over 50% of total terrestrial solar radiation energy, its influence on human skin is not well explored. From a previous study there is evidence that IR might enhance the development of non-epithelial skin cancer and possibly of melanoma. The aim of the present study is to evaluate the impact of IR on UVR-induced apoptosis and DNA repair in normal human melanocytes since malignant transformation depends on the balance between these two effects. Melanocytes were exposed to 250 J cm⁻² IRA (780-1,400 nm), 0.4 J cm⁻² UVB (290-320 nm) or both simultaneously. Apoptosis was determined using cell death ELISA and Annexin V staining 24 hours after exposure. UVB-induced DNA damage was detected applying South-Western dot blot analysis using antibodies against cyclobutane pyrimidine dimers 6 hours after exposure. To investigate different pathways of apoptosis we determined activity of caspase-8 (extrinsic apoptotic pathway) and 9 (intrinsic apoptotic pathway). The apoptotic rate was significantly reduced in melanocytes exposed to IRA and UVB compared to cells exposed only to UVB. IRA did not accelerate the repair of UVB-induced DNA damage. Since DNA damage is a trigger of the UVR-induced intrinsic pathway of apoptosis, we analysed the activity of caspases 9 and 8. While IRA did not influence UVB-induced activation of caspase 9, IRA decreased UVB-induced activation of caspase-8. Hence, it can be concluded that IRA reduces UVB-induced apoptosis via inhibition of the extrinsic apoptotic pathway. Since the repair of UVB-induced DNA-damage is not altered by IRA, IRA might enhance the survival of severely UVR-damaged melanocytes. IRA thus might contribute to an increased risk of malignant transformation of melanocytes.

204 Lavage of the uterine cavity for early and differential diagnosis of serous ovarian cancer


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205 Demonstration of synergistic activity of beauvericin and sorafenib against cervical cancer cell lines

In former studies, the chemotherapeutical application of beauvericin (BEA), a cyclohexadepsipeptide and secondary metabolite of the fungus Fusarium, was discussed, showing in particular strong effects against the human cervix carcinoma cell line KB-3-1. In the current study we combined BEA with the clinically approved multiple tyrosine kinase inhibitor sorafenib (Sora) in vitro to assess possible synergistic effects of the combination regimen. The cytotoxic activity of BEA and Sora alone or in combination was tested in three cervical cancer cell lines (KB-3-1, Caski, HT-3) by MTT. Moreover, flow cytometric apoptosis assays, cell apoptosis stainings, 3H-thymidine incorporation, cell cycle and Western blot analysis were performed to determine signaling response after drug treatment. Finally, human umbilical vein endothelial cells were used for scratch and tube formation assays. Consistent with synergistic cytotoxic effects of BEA and Sora observed in all three cell lines, increased mitochondrial membrane depolarization and enhanced apoptosis in Annexin-V and Hö/PI stained KB-3-1 cells were detected. Likewise, incubation of KB-3-1 cells with both agents led to a reduction of DNA-synthesis and cell cycle arrest in the G2/M stage after 48h. Furthermore, Western blot analyses displayed complex effects on various MAPK pathway molecules. Tube formation and scratch assays revealed reduced formation of endothelial tubes as well as impaired migration of endothelial cells upon addition of subtoxic concentrations of both substances. Our results clearly demonstrate that BEA and Sora exhibit synergistic effects in vitro on several parameters indicative for cancer cell survival, suggesting that BEA might improve the therapeutic activity of Sora in cervix cancer. To confirm the potential clinical relevance of these findings, xenograft experiments with KB-3-1 cells in CB-17 SCID mice are planned to assess the therapeutic effects of BEA and Sora in vivo.

206 Unraveling synthetic lethal interactions of BAF complex mutations and chromatin factors

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SWI/SNF complexes, such as BAF, play important roles in nucleosome mobilization and chromatin remodeling and many of their subunits are recurrently mutated in many different types of cancer. The promiscuous interactions of these complexes with transcription factors, co-activators, and co-repressors modulate numerous signalling pathways. Since most of the mutations of BAF subunits result in loss of function, they represent poor drug targets and the identification of specific vulnerabilities conferred by these mutations is of utmost importance. As previously shown by other groups, targeting putatively mutually exclusive subunits in the BAF complex, such as ARID1B in an ARID1A deficient context or
SMARCA2 in cell lines deficient for SMARCA4, results in synthetic lethality. Focusing particularly on synthetically lethal interactions of the mutated BAF-complex and other chromatin factors, we have performed an RNAi screen comprising 1800 shRNA constructs targeting approximately 400 genes involved in epigenetic functions in 8 different cancer cell lines, either deficient for ARID1A or SMARCA4.

207 Effects of the leukemia-associated C/EBPα p30 isoform on chromatin modulation and transcription

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The gene encoding for the transcription factor C/EBPα is mutated in 9% of patients with acute myeloid leukemia (AML). Use of different ATG codons leads to expression of two C/EBPα isoforms: a full-length version, termed p42, and a shorter form, termed p30. In AML patients, mutations in the N-terminus lead to loss of p42 expression without affecting the p30 coding region. A balanced ratio of C/EBPα isoforms is crucial for hematopoietic homeostasis, as ablation of p42 increases cell growth and blocks myeloid differentiation, resulting in development of AML. However, it is incompletely understood how C/EBPα p30 exerts these leukemogenic effects. It has recently been shown that p30 is a gain-of-function allele, as it displays specific molecular properties that allow it to actively change the gene expression pattern of cells. We hypothesize that these changes occur through p30-dependent modulation of the epigenome. We will study this using two newly developed experimental systems that feature endogenous p30 expression at physiological levels. First, we established and characterized a pre-leukemic myeloid progenitor cell line from fetal livers of Cebpα p30/p30 knock-in mice. Doxycycline-inducible shRNA-mediated p30 down-regulation leads to proliferation arrest and induction of myeloid differentiation of these cells. Secondly, we have modelled AML patient mutations in the N-terminus of the CEBPA gene in the human HL-60 cell line via the CRISPR/Cas9 technology. This is the first description of a human AML cell line harboring N-terminal CEBPA mutations. We will use these novel tools to study effects of p30 on gene expression and chromatin modification by RNA-seq and ChIP-seq. Novel findings will be validated in mouse models of C/EBPα-dependent AML and primary samples from human patients with C/EBPα mutant AML. In conclusion, we expect to gain new insights into the molecular mechanisms through which p30 contributes to AML development.

208 Dichotomy of transforming growth factor- β² signaling in hepatocellular carcinoma progression


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Introduction: Transforming growth factor (TGF)-β is a ubiquitously expressed cytokine with fundamental roles in cell physiology. In carcinogenesis, TGF-β signaling suppresses the proliferation of epithelial cells at early stages by inducing growth arrest and apoptosis. At later stages it triggers epithelial to mesenchymal transition (EMT) and metastasis. To unravel the molecular mechanisms underlying this 'TGF-β-switch' we mimic the pathophysiology situation exposing the cells to TGFβ for longer time. We aimed to identify those cooperating factors and signaling pathways that cause HCC cells to interpret the TGF-β signal in a tumor progressive way. Methods: In vitro comparison of migratory behaviour of various HCC cell lines treated long-term (> 10 days) with TGF-β. Analysis of regulatory networks and target genes. Results: HCC cells that have undergone EMT secrete TGF-β and show elevated levels of Smad2/3 phosphorylation indicating an autocrine regulatory feedback loop. Inhibition of TGF-β abrogates autocrine stimulation and diminishes the migratory potential. Silencing of TGF-β R1 or Smad4 indicated the importance of canonical TGF-β/Smad signaling in cell migration. Short-term treatment of cells with TGF-β could not improve migratory abilities. Interestingly, long-term treatment revealed crucial differences between mesenchymal HCC cell lines. While HLF cells showed increased migration when treated with TGF-β for more than 10 days, SNU449 displayed a reduction in migration. However, both cell lines displayed no modulation in Smad phosphorylation, indicating a change in the utilization of TGF-β signaling in long-term treated SNU449 cells. Conclusion: EMT-transformed HCC cells establish an autocrine TGF-β loop which stimulates migration. However, TGF-β cannot amplify the autocrine loop but causes a different reaction. Interpretation of long-term TGF-β signaling depends on duration and intensity and is controlled by co-acting factors and signaling pathways.
EGFR in healthy well-oxygenated tissues, while the release of the active drug would be selectively triggered under hypoxic conditions inside the tumor. In the here presented study the hypoxic activation and subsequent EGFR inhibition was proven in cell culture. Furthermore, the potent anticancer activity was demonstrated in subsequent in vivo experiments using xenograft mouse models. In addition, the cellular mechanisms involved in the hypoxic activation and EGFR inhibition were further investigated. Thus, the impact of oxygen concentration on the hypoxic prodrug activation was tested by MTT assay. The kinetics of hypoxic activation was also assessed using live-cell microscopy. In order to investigate whether clinically relevant combination strategies would be of interest for the novel hypoxia-activated EGFR inhibitor, several combination protocols were tested in cell culture. Indeed, KP2334 revealed strong synergistic activity with VEGFR inhibitors in these experiments. Overall, our study shows that KP2334 is a promising new drug candidate to improve the tolerability of EGFR-inhibition therapy, which should be further developed in (pre)clinical studies.

210 Evaluation of anti-cancer activity and quality control of the Chinese herbal medicine *Panax quinquefolius* saponin

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*Panax quinquefolius* (American ginseng) is one of the most commonly used herbal medicines in Asia. *P. quinquefolius* saponins (PQS) have been shown to have various pharmacological activities, such as anti-tumor effects. The active components in PQS extracts are considered to be ginsenosides. The aim of this study is to develop the methods for quality control of PQS extracts in accordance with European standards and to evaluate the anti-cancer activity in human cancer cells. An efficient Ultra-High Performance Liquid Chromatography coupled with quadrupole Time-of-Flight Mass Spectrometry (UHPLC-QTOF-MASS) method was developed to analyze the ginsenosides in PQS. Heavy metals, lead, cadmium and mercury were determined using the German Accreditation Office methods. The human prostate cancer cell line DU145 and prostate epithelial cell line PNT2 were used to evaluate the anti-cancer activity of PQS in cell culture. Proliferation was evaluated. We identified the active ginsenosides to be Rb1, Rb2, Rb3, Rc, Rd, Re and Rg1 in PQS extracts. UHPLC-QTOF-MASS experiments showed similar chromatographic results among the samples from six batches, indicating that PQS have good drug stability and quality between different batches. The levels of the measured heavy metals in all batches
were below the detection limits. The inhibitory effects of PQS in proliferation of the human prostate cancer cell line DU145 and prostate epithelial cell line PNT2 were evaluated, which showed that PQS inhibited the growth of prostate cancer cells. In conclusion, we developed an efficient UHPLC-QTOF-MASS protocol for the quality control of PQS extracts, which showed that PQS has good drug stability and quality. Cell culture experiments suggest that PQS may have the potential to inhibit the proliferation of prostate cancer cells. This work is funded by the BMWF/BMG (GZ 402.000/0006II/6b/2012).

211 Mechanisms of acquired FGFR inhibitor resistance in fibroblast growth factor receptor 1-driven lung cancer


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Identification of oncogenic drivers in lung cancer - the major cause of cancer-related death - led to the development of novel targeted therapeutics such as receptor tyrosine kinase (RTKs) inhibitors (TKIs) targeting EGFR or ALK. Despite initial responses, patients relapse due to resistance development. Fibroblast growth factor receptor 1 (FGFR1) gene amplification was identified as an oncogenic driver in a subgroup of small cell (SCLC) - and non-small cell lung carcinoma (NSCLC). Current clinical trials employing FGFR1 TKIs Ponatinib and Nintedanib show promising results and Nintedanib has been approved for treatment of NSCLC after chemotherapy failure. Nevertheless, therapy failure caused by resistance acquisition occurs. Thus, the aim of this study is to investigate the molecular mechanisms underlying resistance development of FGFR1-driven lung carcinoma against FGFR TKIs. To this end, we established FGFR TKI-resistant cell lines from three FGFR1-driven lung cancer cell models. Analysis of the SCLC model by genome-wide array comparative genomic hybridization (aCGH) yielded a focal amplification of the MET gene locus in the Ponatinib-selected subline. Overexpression of MET was confirmed by whole genome gene expression array. Cytotoxicity assays revealed a synergism between Ponatinib and the MET receptor inhibitor Crizotinib, suggesting MET as a driver of resistance in FGFR1-driven SCLC against Ponatinib. Gene expression arrays also yielded a strong transcriptional upregulation of ABCB1 in the Nintedanib-selected SCLC subline. A high-throughput screen of anticancer compounds resulted in resistance against known substrates for ABCB1. Initial functional drug efflux assays suggest Nintedanib also to be a substrate for this pump, suggesting a causative role for ABCB1 in acquired Nintedanib resistance. Thus, resistance of FGFR1-driven lung cancer against FGFR TKIs might involve activation of alternative oncogenic RTK signals and activation of ABC transporter efflux pumps.

212 Oncogenic signaling and epigenetic deregulation - the function of DNMT1 in NPM-ALK driven lymphomagenesis

DNA methylation is an important epigenetic modification that is essential for gene regulation and development. Aberrant DNA methylation is widely observed in tumors, which can result in genomic instability due to global hypomethylation and silencing of tumor suppressor genes due to hypermethylation of CpG island promoters, respectively. NPM-ALK positive anaplastic large cell lymphoma (ALCL) is a Non-Hodgkin lymphoma of T cell origin. About 80% of ALK positive (ALK+) ALCLs carry a translocation, which generates a fusion between the anaplastic lymphoma kinase ALK and the nuclear phosphoprotein nucleophosmin (NPM1). ALK is a receptor tyrosine kinase (RTK) with a putative role in the development of the nervous system. NPM1 is essential for the constitutive activation of ALK, resulting in deregulation of cellular signaling pathways promoting cell proliferation and survival. Recent work has suggested that ALK signaling can directly impact on epigenetic alterations in tumor cells. There is evidence that the downstream ALK mediator STAT3 can upregulate the methyltransferase DNMT1 and target methyltransferases to promoters, which induces silencing of tumor suppressor genes. Using a transgenic NPM-ALK mouse model we demonstrate that T cell specific deletion of the maintenance methyltransferase gene Dnmt1 can inhibit tumor formation. Furthermore, we show that inhibition of DNA methyltransferases using the DNMT inhibitor 5-Aza-2-deoxycytidine revealed antineoplastic activity against ALCL cells in a xenograft mouse model. Similar results can be obtained in NPM-ALK positive murine and human cancer cell lines, where chemical inhibition as well as genetic deletion leads to cell cycle arrest and apoptosis. These data suggest that aberrant DNA methylation is critically involved in ALK dependent lymphomagenesis and DNMT1 might be essential for the oncogenic potential of NPM-ALK. However, the exact function of DNMT1 for tumor development and progression has to be further analyzed.

213 1,25-dihydroxyvitamin D3 modulates the Wnt pathway in non-malignant colonic cells


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Epidemiological studies suggest a correlation between Vitamin D deficiency and colorectal cancer (CRC) incidence. The majority of sporadic tumors develop from premalignant lesions with aberrant activation of the Wnt/β-catenin signaling pathway. The adenoma cell line LT97 harbors an adenomatous polyposis coli (APC) mutation leading to constitutively active Wnt signaling. In these cells, expression of Wnt target genes leads to increased survival capacity. We hypothesized that 1,25-dihydroxyvitamin D3 (1,25-D3), the active form of vitamin D3, promotes differentiation by modulating β-catenin/T-cell factor (TCF) 4-mediated gene transcription. The effect of dietary vitamin D on colonic Wnt signaling was investigated in
mice fed either with 100 IU or 2500 IU vitamin D /kg diet. We examined the effect of 1,25-D3 on differentiation by measuring alkaline phosphatase activity and analyzed mRNA expression of Wnt target genes by real time qRT-PCR. The impact of 1,25-D3 on β-catenin and TCF4 protein expression was assessed by Western blot and immunohistochemistry. In LT97 cells, 1,25-D3 increased cellular differentiation and reduced nuclear β-catenin levels. Further, 1,25-D3 decreased mRNA expression of the Wnt target genes BCL-2, Cyclin D1, Snail1, CD44 and LGR5. In healthy colon of mice fed with high vitamin D diet, the mRNA levels of Wnt5a and ROR2, that promote degradation of β-catenin, were upregulated whereas β-catenin and TCF4 protein expression were decreased. In conclusion, 1,25-D3 inhibits Wnt signaling even in nonmalignant cells underlining its importance in protection against colorectal tumorigenesis and early tumor progression.
2014  Effects of selective nerve transfers on the mammalian motor unit

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Introduction: Selective nerve transfers (SNTs) have been used extensively for the past decade to treat slow nerve regeneration, neuroma pain and improve prosthetic control. SNTs change the motor unit extensively by connecting motor neurons to new functional targets. Good outcomes have been reported but little is known of the structural and functional effects. This experimental study investigates the effects of SNTs using a high capacity donor nerve on the different motor unit levels. Methods: In rats the ulnar nerve (UN) was selectively transferred to the long head of the biceps after neurotomy of the biceps motor branch. After 3, 6 or 12 weeks (each N=15), muscle force and motor unit number estimation (MUNE) were analyzed and both biceps processed for muscle fiber typing. Motor neurons were labeled with Fluoro-Ruby in additional animals with or without SNT (N=17). Results: All SNTs were functional and no dropouts occurred. Muscle force, muscle weight and MUNE increased progressively from 3 to 6 to 12 weeks. At 12 weeks muscle force was 88%, muscle weight 97,5% and MUNE 116,8%, all compared to contralateral control. Retrograde labeling showed 172,3% motor neurons compared to control (p= 0.006; t test). 18,75% of the UN’s motor neurons innervated the muscle after 12 week. Muscle fiber types changed progressively from predominantly intermediate to slow and fast, similar to muscles innervated by the UN. Conclusion: This study shows the course of reinnervation and good functional outcome after a SNT using a high capacity donor nerve. The different motor unit composition led to impressive changes on all levels, most interestingly to functional and structural hyperinnervation of the muscle by the ulnar nerve. These analyses give cellular insights on the good clinical regeneration of SNTs and possible improvements for future applications.

215  Effects of steroid hormones on ecto-5'-nucleotidase expression in rat hippocampus

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Ecto-5-nucleotidase (eN) is cell-surface, rate limiting enzyme in the extracellular dephosphorylation of ATP, catalysing the final step of the AMP conversion to adenosine. It has been suggested that eN is a part of complex molecular network which is under the control of sex steroids, and data about the effects of steroid hormones on eN expression are limited. Thus, we examined the effects of gonadal steroid hormone deprivation, induced by ovary removal (OVX) and steroid hormone treatment, on eN expression determined at gene, protein and functional level in the membrane fraction obtained from hippocampus. After ovariectomy, females were submitted to 17α-estradiol (E2α; 33.3 μg/kg), 17β-estradiol (E2β; 33.3 μg/kg) or progesterone (PG; 1.7 mg/kg) repeated treatments for 7 consecutive days. While ovariectomy induced modest up-regulation of eN at the protein level, the rate of eN activity and gene expression remained unchanged in intact and OVX rats. After steroid hormone treatment, E2α was ineffective, whereas ovarian steroids, E2β and PG, induced complex effects at the eN mRNA, protein and function level. Namely, E2β up-regulated mRNA and increased eN activity while significantly decreased eN protein abundance for about 35%. Although similar trends were detected following PG treatment, the changes were not observed at mRNA level. Considering that eN controls the adenosine production, potent endogenous neuromodulator and homeostatic regulator, results presented herein should be considered relevant for hormone replacement therapy. Acknowledgement Supported by Ministry of Education and Science, project No 173044 and 41014.

216 Changes in Physical and Mental Health during Inpatient Orthopedic Rehabilitation

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Background: Physical rehabilitation after orthopedic surgery is vital to regain full or partial physical abilities such as health-related quality of life (HRQoL). Especially amputees when arriving to rehabilitation report many physical deficits. Often not only physical health is impaired but also mental health. The current study aim was to analyze whether the inpatient orthopedic rehabilitation stay would influence the physical and mental health of orthopedic patients, especially amputees. Amputees were compared to non-amputated orthopedic patients who underwent surgeries i.e. in the knee, hip or spine. Differences between the two groups regarding mobility, HRQoL, pain, body-image and depression were analyzed in a pre-post measurement. It was presumed that the overall physical health would increase during rehabilitation stay, whereas mental impairment would therefore decrease. Methods: 100 patients at the Orthopedic Rehabilitation Center Zicksee (Austria) were tested at admission (T1) and discharge (T2) of their consecutive stay between January and December of 2014. The two groups consisted of 50 amputated patients of 50 non-amputated orthopedic patients. Patients of both groups were selected randomly. The mean age was 61.7 years (SD = 13.0). For statistical analyses, descriptive analyses, t-tests such as repeated measures ANOVA were done. Results: Both groups could increase their performances such as their overall physical and mental health status from T1 to T2. As expected, non-amputated
patients had higher results in mobility and body-image compared to amputees. At the same time, they reported lower mental health and a higher pain level than amputees. Amputees improved most of all regarding physical conditions, whereas non-amputees enhanced their mental health.

217 The role of microRNAs in learned safety

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Background: To detect and interpret safety signals and moreover to react appropriately to those signals is crucial for mental health. Learned safety involves learning about signals indicating protection from danger, thus modulating fear responses. The amygdala is a brain region where specific gene expression changes resulting from safety learning have been observed in mice. The aim of the present study is to try to understand how gene expression in the amygdala during learned safety is regulated focusing on the role of microRNAs. Methods: In a first experiment, expression levels of 11 miRNAs were analyzed in amygdala tissue of learned safety and learned fear trained control mice by qRT-PCR. In a second experiment, expression of the same 11 miRNAs was analyzed in mice trained in a learned fear paradigm and compared to tone alone and shock alone controls. Finally, a bioinformatical scan was performed, to search for potential microRNA target genes. Results: Amygdala expression of 5 miRNAs has been found to differ significantly between learned safety and learned fear trained animals. No differences in the expression of these miRNAs were found between learned fear and tone-alone and shock-alone control groups. A bioinformatical scan revealed various potential target genes for the miRNAs with learned safety specific expression related to stress and depression. Discussion: The selective modulation of expression of 5 specific miRNAs in the amygdala following learned safety suggests that these miRNAs may account for the regulation of various target genes forming the molecular basis for the neural mechanisms underlying learned safety.

218 Genetic Dissection of Anxiety Circuits and their Modification by Psychoactive Drugs

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Anxiolytic drugs have long been used in medicine to relieve the symptoms of anxiety and panic disorders. While the molecular actions of these compounds have been studied in great detail, little is known as to how they alter neuronal processing on the circuit level and how this in turn leads to the observed behavioral phenotype. To address this problem we searched for hot-spots of neuronal modulation after application of benzodiazepines (BZD), a widely used and well studied class of anxiolytic drugs. Using c-fos expression upon BZD administration as a proxy for neuronal activity, we found an increased number of c-
fos expressing neurons in two distinct structures in the mouse forebrain: the paraventricular nucleus of the thalamus (PVT) and the lateral central amygdala (CEl). Within CEl, two interneuronal populations, marked by the expression of either PKC-[delta] or Somatostatin/CRH, are known to gate the output of conditioned fear, which is reduced when the PKC-Δ + population is active. We observed that BZD administration increased c-fos activity predominantly in PKC-Δ + cells, indicating that BZDs might act through this population. Based on these promising preliminary results, we now plan to use pharmacogenetic methods to see whether activity of this population is necessary and/or sufficient for the anxiolytic effects of BZDs. As CEl is further known to receive direct inputs from the PVT, we will use c-fos analysis combined with the injection of a retrograde tracer into CEl to investigate if increased activity is observed in the PVT-CEl projecting neurons. We believe that this would provide a model by which BZDs act on PVT to modulate fear/anxiety gating in CEl. The overarching goal of this work is to provide further insight as to how fear and anxiety are modulated by classic anxiolytic compounds, which will in turn support the development of more specific pharmacological interventions.

219 Antigen availability and the balance between aquaporin 4-specific T cells and NMO-IgG jointly orchestrate lesion location and the extent of tissue damage in the CNS


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Neuromyelitis optica (NMO) is an inflammatory, astrocytopathic disease of the central nervous system (CNS). Ever since the hallmark of this disease - the presence of pathogenic autoantibodies in the serum of most NMO patients - has been recognized, a lot of effort has been made to study how these antibodies reach the CNS to initiate astrocyte-destructive NMO lesions. Since CD4+, activated T cells are found in NMO lesions, and since the classical NMO-IgGs are AQP4-specific immunoglobulins of the subgroup G1, it was tempting to speculate that AQP4-specific T cells might also be responsible for lesion formation in NMO. However, so far, only weakly pathogenic AQP4-specific T cells have been described. We show here that highly pathogenic, AQP4-peptide specific T cells exist, which recognize AQP4268-285 as their specific antigen and cause severe panencephalitis. These T cells are re-activated behind the blood-brain barrier and deeply infiltrate the CNS parenchyma of the optic nerves, the brain, and the spinal cord, while T cells with other AQP4 peptide specificities are essentially confined to the meninges, due to the low local availability of their antigen precluding further T cell activation and parenchymal
immigration. Although AQP4268-285-specific T cells are found throughout the entire neuraxis, they have NMO-typical “hotspots” for infiltration, i.e. periventricular and periaqueductal regions, hypothalamus, medulla, and the dorsal horns of spinal cord. Most remarkably, in the presence of NMO-IgG, they initiate large astrocyte-destructive lesions which are located almost exclusively in spinal cord gray matter.

**220 Phosphorylation of Kv7.2 regulates its PIP2 sensitivity**

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Kv7 channels are a subfamily of voltage-gated K+ channels that play a major role in the regulation of neuronal excitability. One important factor in the function of these channels is PIP2, which is required for gating. GPCRs govern Kv7 channels by determining the levels of PIP2, on one hand, and via phosphorylation on the other hand. However, an interaction of these pathways has not been explored. By applying liquid chromatography-coupled mass spectrometry to Kv7.2 immunoprecipitates of rat brain membranes and heterologous cells, we located a cluster of phosphorylation sites in one of the PIP2-binding domains. To evaluate the effect of phosphorylation on PIP2-mediated Kv7.2 current regulation, we generated a quintuple alanine mutant of according serines (S427/436/438/446/455; A5 mutant) to mimic a dephosphorylated state. Activation of the voltage-sensitive phosphatase Dr-VSP was used to reduce PIP2 levels. Perforated patch-clamp recordings showed that the Kv7.2 A5 mutant needed longer VSP activation time for current inhibition than the wildtype channels. In vitro phosphorylation assays with the purified C-terminus of Kv7.2 revealed that various kinases are able to phosphorylate these 5 serines. After treatment of cells expressing wildtype Kv7.2 with inhibitors of PKA, p38MAPK and CDK5, activation of VSP had to be significantly longer than in untreated controls in order to achieve current inhibition. Our results reveal that the phosphorylation status of residues located within the putative PIP2-binding domain determines the phospholipid sensitivity of Kv7 channels.

**221 Towards investigating the mechanism of action of Paracetamol**

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Paracetamol/ Acetaminophen (APAP) is a widely used analgesic, well characterized in its risk-benefit profile without a clearly elucidated mechanism of action. Paracetamol is mainly eliminated by glucuronidation and sulfation, while some of it is converted into a reactive intermediate, NAPQI (N-acetyl-p-benzoquinone imine) by cytochrome P450 enzymes. The M current is characteristic of the neuronal subtypes of voltage-gated potassium channels (Kv7 family). Inhibition of M currents is linked to enhanced neuronal excitability while their augmentation causes neuronal silencing, with established translational use in pain management and epilepsy. The project aims at understanding if NAPQI is involved in the antinociceptive action of paracetamol. Our preliminary data show a progressive enhancement of M current in heterologously expressed Kv7.2 homomers starting at 0.3 µM NAPQI with a threefold rise at 10 µM, which is maintained during a control washout for 5 minutes. The Kv7.2/7.3 heteromers and capsaicin positive DRG neurons showed a similar profile with a maximal response at 0.3 µM and 1 µM NAPQI respectively and a decrease in the amplitude of current during washout. Furthermore, the excitability of capsaicin positive DRG neurons is reduced on application of 10 µM NAPQI. These results indicate that the mechanism of action of paracetamol could be explained by the enhancement of M current and consequently a decrease in excitability of DRG neurons by its metabolite NAPQI.

Maternal immune activation epigenetically regulates expression of the serotonin transporter in the adult offspring brain

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Major depressive disorder (MDD) is one of the most debilitating diseases worldwide, yet the underlying pathological mechanisms are poorly understood and some patients are not adequately treated. Recently, involvement of the immune system has been proposed - both in acute depressive episodes and as a potent environmental risk factor during neural development in form of maternal immune activation (MIA). Indeed, in a rodent model mimicking gestational infection by MIA through the administration of Poly(I:C) to the pregnant dam, depressive-like behaviour of adult offspring has been reported. However, the underlying molecular mechanisms are only starting to be elucidated. Increasing evidence points towards epigenetic mechanisms as central mediators of the impact of environmental influences on gene expression and thus brain structure and function. Here, we investigated the effect of MIA on molecular participants of epigenetic regulation with a special focus on the serotonin transporter, critically involved in the aetiology of MDD and pharmacological antidepressant treatment, as well as selected epigenetic markers in the hippocampus of MIA offspring. We found a reduction of histone acetylation specifically for H4 in hippocampal tissue of MIA offspring and a selective decrease in levels of histone deacetylases (HDAC) 2 and 9. Both SERT mRNA and protein expression and were significantly reduced in MIA offspring and a significant decrease in H3 acetylation as well as an increase in H4 acetylation at the SERT promoter was observed by chromatin immunoprecipitation. These findings support the notion that epigenetic mechanisms contribute to the environmental programming of brain development and behaviour by embedding the impact of the early life experiences on gene expression. Thus the data
suggests that distinct hippocampal global and gene-specific histone acetylation patterns may ingrain the effects of MIA on SERT expression and depression-like behaviour later in life.

223 The role of H2S in autonomic nervous system

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Hydrogen sulfide (H2S) is a toxic gas also produced in mammalian tissues where it can exert various functions as gasotransmitter, such as opening of smooth muscle KATP channels and resulting vasorelaxation. A recent study showed that H2S is endogenously generated and released in sympathetic ganglia and potentiates ganglionic transmission (Sha et al., 2013). In primary cultures of rat superior cervical ganglion (SCG), we found that in radiotracer release experiments, basal tritium overflow as well as outflow triggered by either electrical fields or depolarizing K+ concentrations were enhanced by 0.1 to 1 mM of the H2S donor NaHS in a concentration-dependent manner. In electrophysiological experiments, H2S hyperpolarized the SCG membrane potential and reduced action potential firing. In SCG neurons, hyperpolarisation of membrane potential can be caused by an enhancement of currents through Kv7 channels (Lechner et al., 2003). Unexpectedly, NaHS inhibited currents through Kv7 channels in a concentration-dependent manner, whether endogenously expressed in SCG neurons or heterologously expressed in tSA cells. Diazoxide, a well known KATP channels opener, also hyperpolarized the SCG membrane potential leading to the hypothesis that the membrane hyperpolarization caused by H2S could be an effect mediated by KATP channels.

224 Cav1.4 IT mouse model for congenital stationary night blindness type 2 and its retinal morphology

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The L-type calcium channel \( \text{Ca}_{1.4} \) is predominantly expressed in the photoreceptor synapse and there is crucial for neurotransmitter release. Mutations in the encoding region CACNA1f are linked to congenital stationary night blindness type 2 (CSNB2). Patients show abnormalities in their electroretinogram (ERG). Previous functional analyses in a heterologous expression system revealed a marked leftward shift in activation, indicating a reduced dynamic range of the retina. ERG of \( \text{Ca}_{1.4} \) IT mice also showed a loss in rod answer and diminished cone answer. For a better understanding of the pathomorphology of the retina, 10-week old mice carrying the \( \text{Ca}_{1.4}\text{I745T (IT)} \) mutation were investigated. Retinal thickness was severely reduced. Cones were shortened and the axons manifested knots and swellings. The synaptic terminals of cones (pedicles) were enlarged and displaced towards the outer nuclear layer. Photoreceptor ribbon synapses possessed an immature morphology which is in agreement with a role of \( \text{Ca}_{1.4} \) in synaptic development and maturation. Horizontal and bipolar cells showed elongated dendrites extending into the ONL. The inner retina appeared only mildly unaffected by the \( \text{Ca}_{1.4} \) IT mutation. Calbindin D28k staining, which labels three strata in the inner plexiform layer, revealed a similar appearance in wt and IT retinas. The gap junction protein connexion 36 showed decreased expression in the OPL as well as an impaired expression pattern in the ON and OFF sublayers of the IPL. The expression of metabotrophic glutamate receptor 6, which in wildtype is localized to ON bipolar cells, was reduced to background in the IT retina. Overall the integrity of the outer plexiform layer was found disorganized, which is in agreement with a role of \( \text{Ca}_{1.4} \) in synapse development and maturation. This study was funded by the FWF-Support (P22526), SFBF44020 and the Medical University Vienna.

225  LTP reinforcement in rat hippocampus in the presence of spatial behavioural stimuli

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Hippocampus serves as a complex memory processing system. Hippocampal dependent LTP reinforcement takes place in the presence of a motivational context. Protein synthesis functions as a biochemical basis of the functional plasticity, like LTP. To investigate the role of presence of behavioural stimuli in hippocampal-LTP reinforcement, we performed hole-board behavioural test in-vivo. The field excitatory postsynaptic potential (fEPSP) and the population spike amplitude (PSA) were recorded in the laminar moleculare and the granular cell layer, respectively. Electrodes were implanted into the dentate gyrus of the hippocampal formation of the right hemisphere. The bipolar stimulation electrode was implanted in the medial perforant path. Transformation in LTP was observed in the presence of the behavioural stimuli and the reinforced LTP was persistent for 6 hr. Given the effects in presence of behavioural stimuli in LTP reinforcement, the possible variations in the membrane protein receptor levels of the dorsal hippocampus were investigated. The glutamatergic and monoaminergic membrane protein levels were determined using native electrophoresis and subsequent analysis by quantitative western blotting. Among the glutamatergic membrane receptors we checked, GluN1 and GluN2A -
containing NMDA receptor, GluA1 and GluA2-containing AMPA receptor levels and the monoaminergic Dopamine (1A) receptor levels were remarkably and significantly higher in the animal group that received behavioural input in comparison to the control group. However, a pronounced change was observed with the GluA3-containing AMPA receptor levels and monoaminergic 5HT1A receptor levels, were significantly decreased in comparison to the control group. In conclusion, our results suggest, these protein syntheses and their association (a heterosynaptic association) in the hippocampus might contribute to or mediate the reinforcing effects of LTP in the presence of spatial behavioural stimuli.

226 Laser-capture microdissection assisted detection of somatic cancer genes mutations in skeletal muscles from mdx mice: linking muscular dystrophy to cancer

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Duchenne muscular dystrophy (DMD) represents a rare incurable disease, which is characterized by loss of functional muscle tissue due the absence or vast reduction of dystrophin. In mdx mice, a model for DMD, our group observed a high susceptibility for skeletal muscle associated malignant sarcomas, which frequently harboured recurrent mutations in known cancer genes. Thus, we hypothesized the existence of these sarcoma-associated mutations in dystrophic muscle and postulated that cell clusters residing between dystrophic fibres, a common pathological feature, might represent early microscopic sarcomas.

We investigated these putative sarcoma pre-stages in dystrophic muscles from mdx mice without clinically overt sarcomas. Using laser-capture microdissection, cell clusters residing between muscle fibres were isolated from muscle cross-sections and subjected to genetic analysis by quantitative PCR, targeting known loci of recurrent mutations in MD-associated sarcomas (oncogenes Met and Jun, tumour suppressors Cdkn2a and Nf1, duplication of chromosome 8 and/or 15). Muscles from n=15 mdx mice (300-750d old) were screened via consecutive H&E-stained cross-sections. Out of n=71 samples, n=13 muscles contained cell clusters suitable for analysis. PCR analysis revealed that cell clusters harbour a subset of sarcoma-associated mutations, namely deletions in the Cdkn2a and Nf1 genes, which were identified at a frequency of 23% and 15%, respectively. In contrast, neither amplification of Met or Jun, nor chromosome 8/15 copy number alterations were detectable. We were able to identify cancer gene mutations in dystrophic muscles from mdx mice without clinically overt sarcomas. The occurrence of Cdkn2a and Nf1 deletions suggests that loss of these tumour suppressors might probably constitute early events in sarcoma formation. Our results further demonstrate that cell clusters residing between muscle fibres indeed might represent sarcoma pre-stages.

227 Functional characterization of cytoskeletal proteins in neuromuscular synapse formation using an inducible RNAi approach in muscle cells
Muscle specific kinase MuSK plays an essential role in formation and maintenance of the neuromuscular synapse (NMS). MuSK becomes autophosphorylated and initiates its kinase activity in response to neural agrin. Activated MuSK phosphorylates downstream targets to induce a signaling cascade driving presynaptic and postsynaptic differentiation characterized by the clustering of acetylcholine receptors (AChRs). Impaired MuSK function results in acute neuromuscular deficiencies as shown in myasthenia gravis or to perinatal death in MuSK deficient mice due to respiratory failure.

We have used a quantitative mass spectrometry method to identify and investigate the phosphoproteomic map of MuSK signaling. We identified 203 regulated phosphopeptides, which were classified into 4 different clusters according to their temporal profiles. Interestingly, we detected an overrepresentation of proteins involved in cytoskeletal rearrangements such as Vinculin, Paxillin and LL5β, which have previously been shown enriched and functional at developing AChR clusters. Based on that and also due to the indispensable role of the cytoskeleton in NMS formation, we have focused on regulated phospho-targets with cytoskeletal functions including Palladin, Paxillin, focal adhesion kinase. Our aim is to silence targets in differentiated myotubes using RNAi and to determine their role during MuSK signaling, AChR clustering and NMS formation. To downregulate the expression of targets in differentiated myotubes and not in proliferating myoblasts we have developed an inducible miRNA-based knock-down system in muscle cells. Silencing efficiency of our approach was confirmed by downregulating MuSK, which resulted in a failure of AChR clustering as expected. Then, we have generated muscle cells that allow an inducible downregulation of selected phospho-targets to assay their role during MuSK signaling and clustering. We expect that these studies help to discover novel proteins involved in NMS formation.

228 The environment modulates the conformation of transmembrane helix 1A in the Leucine Transporter (LeuT)

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Neurotransmitter transporters are found on the presynaptic neurons and on glial cells. The function of these transporters is termination of neurotransmission by rapid removal of neurotransmitter molecules from the synaptic cleft. These transporters couple substrate transport to the ion gradients of sodium and chloride. Structural studies often require the transporter to be removed from its physiological membrane, which can affect its structure or conformation. Crystal structures of the bacterial homolog LeuT (SLC6 family) were solved in three states of the transport cycle: occluded, outward and inward. The recent inward facing structure shows a conformation where the first helix (TM1A) did not seem to be compatible with the membrane environment. We carried out molecular dynamics simulations of LeuT in...
membrane and micelle environment to investigate the conformational behaviour of TM1A and combined the investigation with distance measurements using LRET. We used POPC as membrane lipids, and build the micelle systems with three different protein-detergent ratios (1:120, 1:140, or 1:160) using the detergent n-Octyl-β-D-Glucopyranoside (BOG) molecules. We observed a rigid body motion of the TM1A helix: it moves out of the hydrophobic core of the membrane. In contrary, TM1A was stable in its position in the micelle simulations. We confirmed this observation by distance measurements of solubilized LeuT in micelles and reconstituted POPC liposomes. This study suggests that the polar part of helix TM1A would not protrude into the membrane core. To further characterize the conformation of the TM1A helix, we used SMD simulations and pulled this helix relative to the scaffold or the core domain. The free energy profile was in line with our findings in the position of TM1A. This study indicates that changes in the environment can affect the equilibrium conformation of LeuT.

**Multiple Sclerosis: Investigating affective and higher cognitive Theory of Mind**


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Theory of Mind [ToM], the ability to represent mental states of oneself and others, can be divided into cognitive and affective ToM. Research shows inconsistent results regarding ToM impairments in patients with Multiple Sclerosis (MS), research regarding higher order ToM in MS is scarce. The aim of the study was to investigate affective and higher order cognitive ToM in MS. A sample of 38 patients (22f, 16m; disease duration: M=11.1 years, SD=6.1; EDSS: Median=1.5) with relapsing-remitting (n=35) or secondary progressive (n=3) MS aged 22-65 years (M=40.6; SD=9.7) and 38 gender-, age-, and education-matched healthy controls took part. Cognitive ToM was investigated using six ToM-Stories, affective ToM was investigated using the Reading the Mind in the Eyes Test (RMITE). No group differences emerged regarding cognitive ToM total score (T=-1.182, p=.245), lower order ToM (1st-order: T=-1.055, p=.298; 2nd-order: T=-.794, p=.458) and higher order ToM (3rd-order: T=-1.091, p=.282). Regarding cognitive ToM total score patients (T=-3.597, p=.001) and controls (T=-3.822, p=.0001) showed sign. increasing scores in the course of the study. Regarding the first block patients were sign. slower than controls (T=3.608, p=.001) for the latter there was no difference (T=1.346, p=.187). Only patients showed a sign. increase (T=4.1, p=.0001). Regarding affective ToM no differences could be found (T=-1.151, p=.257). Results showed no group differences in ToM performance hinting that MS leads to network impairments permitting compensatory mechanisms. For cognitive ToM learning effects could be found for both groups. At the beginning controls showed significantly shorter conduction times with these differences subsequently disappearing. These results suggest that in MS sufficient mental flexibility and the potential for learning and improvement of information processing efficiency is still given. This study is funded by a grant of the Austrian MS Forschungsgesellschaft.

**Oppositional COMT Val158Met effects on resting state functional connectivity in adolescents and adults**
Prefrontal dopamine levels are relatively increased in adolescence compared to adulthood. Genetic variation of COMT (COMT Val158Met) results in lower enzymatic activity and higher dopamine availability in Met carriers. Given the dramatic changes of synaptic dopamine during adolescence, it has been suggested that effects of COMT Val158Met genotypes might have oppositional effects in adolescents and adults. The present study aims to identify such oppositional COMT Val158Met effects in adolescents and adults in prefrontal brain networks at rest. Resting state functional connectivity data were collected from cross-sectional and multicenter study sites involving 106 healthy young adults (mean age 24 ± 2.6 years), gender matched to 106 randomly chosen 14-year-olds. We selected the anterior medial prefrontal cortex (amPFC) as seed due to its important role as nexus of the executive control and default mode network. We observed a significant age-dependent reversal of COMT Val158Met effects on resting state functional connectivity between amPFC and ventrolateral as well as dorsolateral prefrontal cortex, and parahippocampal gyrus. Val homozygous adults exhibited increased and adolescents decreased connectivity compared to Met homozygotes for all reported regions. Network analyses underscored the importance of the parahippocampal gyrus as mediator of observed effects. Results of this study demonstrate that adolescent and adult resting state networks are dose-dependently and diametrically affected by COMT genotypes following a hypothetical model of dopamine function that follows an inverted U-shaped curve. This study might provide cues for the understanding of disease onset or dopaminergic treatment mechanisms in major neuropsychiatric disorders such as schizophrenia and attention deficit hyperactivity disorder.

231 Transdifferentiation of fibroblasts from patients with Rett syndrome into neuronal progenitor cells via episomal transduction of neuronal transcription factors

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INTRODUCTION: Rett syndrome is a severe neurological disorder caused by mutations in the X-linked gene coding for the methyl CpG binding protein 2 (MeCP2). Since human studies on cells from Rett patients are still missing, this project aims to transdifferentiate human Rett fibroblasts into neuronal
progenitor cells via episomal transduction of the neuronal transcription factors Pax6 and Sox2. METHODS: The cDNA of the two transcription factors Pax6 and Sox2 was cloned into the EEV600A-1 episomal vector. Plasmid DNA containing Pax6 and Sox2 or Sox2 alone were transfected into fibroblasts of a male Rett patient carrying a MeCP2 mutation using electroporation or chemical transfection. The transfection efficiency and the survival rate were monitored by immunofluorescence (IF) using Pax6 and Sox2 antibodies, 3 days after transfection. The medium was changed to neural precursor proliferation medium at the same time point. The morphological changes during the reprogramming process were documented in phase contrast microscopy on every other day over a period of 6 - 11 weeks. RESULTS: Three days after electroporation 10 - 25% of the cells were positively stained in IF microscopy for the expression of Sox2 and Pax6. Chemical transfection led to an efficiency of 5 - 10%. The cell density usually decreased during the repogramming process and the earliest induced neural progenitor (iNP) colonies appeared 30 days after transfection in some of the experimental approaches. Surprisingly, the transfection efficiency did not correlate with the efficiency of transdifferentiation. CONCLUSION: The transfection of two episomal plasmids coding for Pax6 and Sox2 or the transfection of Sox2 alone into fibroblasts of a male patient with Rett Syndrome led to the formation of early iNP colonies after cultivation in neural progenitor proliferation medium for several weeks. The identity of these colonies has to be confirmed by analyzing the expression of NP markers in q-RT-PCR.

232 Firing patterns of distinct types of principle cells in the medial prefrontal cortex during working memory and rule switching task

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Synchronous activity of neuronal networks is a fundamental requirement for precise transmission of information to drive behavioral responses. In cerebral cortex, distinct types of neuron contribute differentially to network activity to establish a spatiotemporal division of labor. To date, despite the continuous efforts to explain cortical events through specific circuits, we still lack the basic knowledge of how many types of neuron exist and how these distinct cells are interconnected. In medial prefrontal cortex, excitatory principle cells extend axons to far distant intracortical, subcortical and subcerebral targets. Across different cortical layers, principle cells have diverse projection profiles, with unique axo-dendritic arborisations, expressing different transcription factors and ultimately serving different network operations. By using juxtacellular recording/labelling technique, tetrode and chronic silicon probes, we recorded single and large-scale multiple unit activity of the medial prefrontal cortex in freely behaving rats during a working memory and rule switching task to understand the diversity of principle cells, their interactions with interneurons and how they contribute in the local circuitry to drive highly precise working memory and cognitive flexibility.
How to rescue sleepless fly: pharmacochaperoning by noribogaine restores sleep in *Drosophila melanogaster* harboring mutated dopamine transporters.

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The dopamine transporter (DAT) belongs to the solute carrier 6 gene (SLC6) family. DAT is a transmembrane protein, which is delivered to the presynaptic specialization, where it accomplishes its eponymic function: it retrieves dopamine from the synapse and operates in relay with the vesicular monoamine transporter to replenish vesicular stores. Mutations in the coding sequence of the dopamine transporter gene lead to misfolding of the transporter. The resulting retention of DAT in the ER causes infantile dystonia/Parkinsonism. In *Drosophila melanogaster*, mutations, which abrogate the function of drosophila DAT (dDAT) lead to sleepless phenotype. We identified one mutation, i.e. dDAT-G108Q, which may arise from folding deficiency based on the following arguments: (i) dDAT-G108Q is a loss of function mutation. (ii) The glycine residue in the position corresponding to G108 in dDAT is absolutely conserved in all species from *C. elegans* to *H. sapiens*. (iii) G108 is at the end of the intracellular loop 1, which is important for folding of SLC6 transporters. We verified this conjecture by examining the cellular distribution of dDAT-G108Q upon expression in both, human HEK293 and drosophila Schneider cells. Confocal microscopy confirmed that dDAT-G108Q was retained within the endoplasmic reticulum. Surface expression was restored upon preincubation of the cells with noribogaine and pifithrin-μ. This pharmacochaperoning action of noribogaine is accounted for by its ability to bind to and stabilize the inward facing conformation of DAT. The pharmacochaperoning action of pifithrin-μ is due to its ability to block HSP70. We also examined, whether these two compounds restored sleep in dDAT-G108Q expressing flies by including the compounds in the food: this was the case. Thus, the experiments provide a proof-of-principle that mutations in DAT are amenable to rescue by pharmacochaperoning in vivo.

Transcendence, religion and spirituality in medicine - Austrian medical students' point of view.

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Spirituality concerns the search for transcendent meaning, whereas religion is belief in a defined transcendent power. Illness touches on the transcendent meaning in patients' lives. A large number of studies have shown that religion and spirituality may play an important role in patients' life while dealing with serious illness. Data demonstrated the existence of a “spiritual need” in patients and the importance of addressing spiritual issues in patient- doctor communication. But the role of patients' spirituality and religion still seems to be a taboo in the daily routine of a doctor. Do we, as physicians, neglect these important issues? Are we ready to confront ourselves with our own religious/spiritual beliefs and furthermore to open doors for our patients to talk about it? Do physicians of tomorrow, medical students, believe that the “faith factor” plays a role while coping with illness? Do they feel comfortable to integrate religion/spirituality into their patients’ care? To find answers for precisely these questions 1400 students (656 female and 744 male) at the Medical University of Vienna participated in a questionnaire, between March and June 2013. This questionnaire is assessing the students’ spirituality and religiosity, as well as their attitudes towards “spiritual care”, particularly to its relevance in their future profession. 59.5 % of the students had reflected their own belief concepts, 21.9 % consider themselves as religious, and 20.1 % as spiritual individuals. 75.6 % of the students agreed with the statement that religious conviction/spirituality might have an effect on cancer patients’ coping. 85.9 % will consider talking with their patients about religious/spiritual issues, if patients wish to do so. 86.3 % would involve chaplains if they had the feeling it is necessary. The results of this study encourage the view that future doctors should see the patient in a wider scope than the bio-psycho-social one, by including the spiritual dimension.

235 Add-on pharmacotherapy with antipsychotic drugs in patients with obsessive compulsive disorder and non-response to monotherapy with serotonin reuptake inhibitors

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Background: Treatment resistance to serotonin reuptake inhibitors (SRIs) represents a critical issue in the pharmacological management of obsessive-compulsive disorder (OCD). The administration of antipsychotic drugs in addition to the ongoing SRI treatment can be beneficial in this regard and our meta-analysis sought to elucidate the current evidence for this frequently applied treatment strategy. Methods: We conducted a meta-analysis of double-blind, placebo-controlled, randomized controlled trials (RCTs) that compared augmentation of SRIs with antipsychotics to placebo supplementation in treatment-resistant OCD. In order to identify all relevant trials, we performed a systematic literature survey in various electronic medical databases. Primary outcome was mean reduction in the Yale-Brown Obsessive Compulsive Scale (Y-BOCS) total score. Secondary outcomes were dichotomous response and
drop-out rates. Hedges` g and risks ratios were estimated as continuous and dichotomous effect sizes. Results: After the accomplishment of the systematic literature search, we could include a total of 14 relevant RCTs (n=491) examining quetiapine, risperidone, aripiprazole, olanzapine, paliperidone, and haloperidol were investigated. The antipsychotics were significantly superior to placebo in Y-BOCS total score reduction (Hedges` g=-0.64, 95% CI: -0.87 to -0.41; p<0.01). Aripiprazole, haloperidol, and risperidone were significantly more efficacious as compared to placebo. These superiorities were paralleled by a significant superiority of the antipsychotic augmentation when analyzing dichotomous response rates. There were no significant between-group differences for the drop-out rates. Conclusions: Adding antipsychotic drugs to SRIs in treatment-resistant OCD antipsychotics can be regarded as evidence-based treatment approach in therapy-refractory OCD.

236 Lateralization of language function in epilepsy patients: An event-related potential (ERP) study of a visual word memory/recognition task


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Introduction: Assessment of language lateralization is important in epilepsy surgery to avoid postoperative language deficits. Although the Wada test is considered the gold standard for language lateralization, non-invasive substitutes such as fMRI represent the current clinical standard, but show discordance rates of ~15%. Event-related potentials (ERPs), especially the language-related negative component around 400ms, are related to language processing and are therefore expected to reflect language lateralization. Method: The study was based on a 2 (Lateralization; left vs. right hemisphere) × 2 (Stimulus; memorized words vs. new words) × 2 (Group; patients vs. controls) repeated ANOVA design with Group as a between factor. Scalp EEG was recorded from 64 standard locations in 27 drug-resistant focal epilepsy patients and 28 healthy controls (all right-handed) during a visually presented word recognition task, where abstract nouns had to be memorized and later recognized from a larger word list. ERP areas of memorized and new words (45 trials each; randomly presented; stimulus presentation time 1000ms; ISI 2700-3200ms) were analyzed in the 400-600ms epoch. Language fMRI was routinely obtained in epilepsy patients. Results: ANOVA showed a significant interaction of Lateralization × Stimulus × Group (F (1,53)=4.13; p=0.047), indicating a more negative potential for memorized words over the left compared to the right hemisphere, and this was more pronounced in patients compared to controls, whereas no effects appeared for new words. Effects. The individual comparison of the mean ERP area showed a left-sided lateralization in 80% of epilepsy patients. Discussion: ERP of recognition of memorized words were lateralized to the left hemisphere in healthy controls and epilepsy patients. In patients, single-subject laterality indices showed 80% concordance with fMRI results. Results indicate
that scalp-derived ERPs of memorized words are a promising tool to investigate lateralization of receptive language functions and verbal working memory in epilepsy patients.

237 The demographic distribution of using hypnosis in patients

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Introduction: There is growing interest among patients, physicians and healthcare administrators in complementary/alternative medicine (CAM). It has become an important aspect of treatment nowadays. Hypnotherapy is one of the CAM therapy which is considered as a procedure used to treat a variety of symptoms, including pain, anxiety and depression. There are no published data on the distribution pattern of hypnotherapy in Iran. This survey was conducted to explore the demographic distribution of using hypnosis in 100 outpatients referred to the CAM center. Materials & Methods: This is a descriptive cross-sectional study which was performed in CAM center of Healthy life in Iran, from June 2011 – March 2012. The fundamental treatment in this center is Hypnotherapy but depend on each patient some medical treatment were chosen parallel. A retrospective record review was conducted by captured data from records during 18 months. We used the special questionnaire focused on 4 constructs: 1- demographic data, 2- Chief Complaint, 3- hypnosis treatment and 4- outcome. We used SPSS 16 software, Chi-square, Spearman correlation, ANOVA and T-test for analysis (P<0.05). Results: 100 records out of 183 were conducted to our study. The majority of patients were middle aged (59%); the age ranged from 26-45 years. 68% were female and others were male (32%). Married and single patients were almost equal, 44% and 41% respectively. About the educational attained; 64% had well educated (bachelor and above). The majority of them (98%) were right handed and were occupied (58%). Most people (52%) complained about anxiety disorder. 19% had mood disorder. About stop smoking 12% were found. Other problems such as eating/sleep, personality and dissociative disorder were the least complaint was seen (10%, 4% and 1% respectively). Approximately 50% patients were consuming medications for their illness previously. 48% gave complete treatment. Most patients (62%) continued their treatment within more than 1 month interval. 22% patients gave only hypnosis treatment, 78% gave both hypnosis and medications. About the effectiveness of hypnotherapy; 77% said their illness became better or much better/controlled than before. 6% believed their complaint has been completely removed. No one complained about getting worse but 17% didn’t experience any changes. There were significant correlation between the complete treatment and less than 1 month treatment interval with good outcome of hypnotherapy (p=0.004 and 0.006 respectively). We found the considerable correlation between good outcome of hypnosis and no medication use (p=0.035). It means that among 77% with good results, patients who had no drug consumptions (48%) got better results versus others (29%) use medications. Another result is the majority of patients (11%) with no changes outcome used medication and 6% have got no drugs. Conclusion: Our study showed use of hypnosis is prevalent in Iran and found that hypnosis is safe and effective in psychiatric disorders.
Role of GABAergic interneurons to prefrontal cortex network activity during a rule-switching task

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The prefrontal cortex is a brain region for strategic planning and executive functions. Operations of the prefrontal cortex is impaired in schizophrenic patients. This leads to various cognitive deficits, notably in cognitive flexibility, the ability to quickly change behavior in a novel situation. Network operations in the prefrontal networks allow the re-evaluation and outcome-predictions in the light of new conditions. In this context, we investigate the contribution of GABAergic interneurons of the prefrontal cortex to behavioural flexibility. Interneurons have highly adaptable firing patterns, contribute to neuronal timing and network synchrony, cortical circuit selection and gain modulation. In the cerebral cortex a large diversity of interneurons exist based on synaptic connectivity, protein expression and firing patterns. However, the respective contribution of distinct types of interneuron to network operations for behavioural flexibility is yet to be assessed. We train rats to perform an extra-dimensional rule-switching task on a Y-maze. Large scale extracellular recordings, using tetrodes or silicon probes, allow us to monitor the activity of neuronal networks engaged during such behavior. This approach is combined with the juxtacellular recording and labelling of interneurons, which allows to determine the firing patterns of unequivocally identified neuron types. Preliminary results will be presented and discussed.

Measuring and manipulating brain activity with concurrent TMS/fMRI


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Transcranial magnetic stimulation (TMS) has emerged as a powerful non-invasive application widely used in therapeutic settings and neuroscientific research. Application of repetitive TMS (rTMS) leads to changes that usually outlast the duration of the stimulation. Combination with neuroimaging methods can provide the missing control by measuring brain activity changes following TMS and can relate it to result behavioral changes. However typical setup presents some limitations, most importantly the poor sensitivity of the required MR birdcage coil. We present new hardware and analyses methods. Experiment 1: New TMS/fMRI coil array. 10 healthy subjects (age: 26.1 ±3.6 f/m:8/2) participated in a
concurrent TMS/fMRI experiment involving stimulation over the hand-area of the motor cortex using the new developed hardware. The stimulation protocol comprised five TMS blocks of each of different intensities. Response in the M1 hand area shows a monotonic increase, with no response at 80% MT stimulation. For the area directly underneath the TMS coil, we found a clear linear relationship between response and stimulation intensity. Experiment 2: Influence of rTMS over DLPFC on RS network. 60 healthy subjects (age: 25.01±4.6 years, f/m: 31/29) underwent rTMS DLPFC and sham stimulation. Resting state fMRI scans were acquired before and twice after stimulation. We investigated BOLD signal changes in functional connectivity (fc) following 10 Hz rTMS during a classic resting state paradigm. Resting state fc was examined before and twice after rTMS. We examined RSFC changes by using an unbiased approach based on a set of 20 well-established resting state networks derived from multicenter - data by Biswal et al. (2010). This study gives proper evidence that rTMS over left DLPFC influences the ACC within a specific network while other networks stay stable. The effect is observable over the whole group, is independent from behavioral changes and no networks were altered by sham stimulation.

240 The norepinephrine transporter in adult attention deficit hyperactivity disorder quantified with (S,S)-[18F]FMeNER-D2 and PET


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Introduction: The norepinephrine transporter (NET) is a central target for often prescribed medications in attention deficit hyperactivity disorder (ADHD). Thus, the NET is suggested to be highly involved in the underlying neurobiological mechanisms in ADHD. The aim of this study was to quantify NET binding using positron emission tomography (PET) and the highly selective radioligand (S,S)-[18F]FMeNER-D2 in adult ADHD. Methods: 22 medication-free ADHD patients (age: 32.16 ± 10.86, mean ± SD, 12 males) without psychiatric comorbidities, and 22 age and sex matched healthy controls (HC, age: 32.00 ± 10.56, 12 males) underwent PET scanning once. The NET binding potential (BPND) was compared between groups by means of a linear mixed model analysis including the hippocampus, the putamen, the pallidum, the thalamus, the midbrain with pons (stating a region of interest (ROI) which includes the LC), and the cerebellum (atlas-based ROIs). Additionally, we included thalamic sub-nuclei (13 atlas-based ROIs) and LC and thalamus (manually delineated ROIs) to the analysis. The 2-tailed significance level was set at 0.05. Results: The results of this investigation revealed no main effect of subject groups (F1,41<0.01,p=0.96, Fig. 1) or sex (F1,41<0.01,p=0.98) and no interaction effects (all p>0.1). Further, we reveal no significant association between ADHD symptoms severity and regional NET availability. However, we found a
significant negative correlation of NET BPND with age in the thalamus ($r= -.54$) and midbrain ($r= -.42$, $p<0.05$ corrected), but these correlations did not differ between HC and ADHD patients. Conclusion: Our findings show no significant differences in the NET BPND of (S,S)-[18F]FMeNER-D2 between ADHD patients and HC. Thus, results imply that NET BPND is not a major player in the pathogenesis of ADHD. However, different molecules of noradrenergic transmission might be altered, or the noradrenergic transmitter system might be affected on different levels, such as cortical regions, which cannot be reliably quantified with this radio-ligand.

241 The combined effect of genetic polymorphisms and clinical parameters on treatment outcome in treatment-resistant depression


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Introduction: With lifetime prevalence estimations reaching 20% major depressive disorder (MDD) ranges among the most burden heavy disease. While clinicians can rely on a repertory of antidepressant agents that is potent to alleviate or cure the symptoms of most patients suffering from MDD, about 30% of these patients are currently not responding to the first antidepressant administered and up to 15% show hardly any relief even after multiple agents were applied1. For over a decade, the European Group for the Study of Resistant Depression (GSRD) has examined single nucleotide polymorphisms (SNP) and clinical parameters in regard to treatment outcome. However, an interaction based model combining these factors has not been established yet. Regarding the low effect of individual SNPs, a model investigating the interactive role of SNPs and clinical variables in treatment-resistant depression (TRD) seems auspicious. Methods: 225 patients featured in previous work of the GSRD were enrolled in this investigation. According to data availability and previous positive results, 12 SNPs in HTR2A, COMT, ST8SIA2, PPP3CC and BDNF as well as 8 clinical variables featured in other GSRD studies were chosen for this investigation2-4. Random forests algorithm were used for variable shrinkage with special regard to interaction effects. Subsequently k-means clustering was applied for surfacing variable characteristics determining treatment outcome. Additionally, the resulting model was challenged by predicting
treatment outcome in an untapped sample of 74 patients also deriving from the GSRD data pool. Results:
Using these machine learning and clustering algorithms, we detected a set of 3 SNPs and a clinical
variable that was significantly associated with treatment response. About 62% of patients exhibiting the
allelic combination of GG-GG-TT for rs6265, rs7430 and rs6313 of the BDNF, PPP3CC and HTR2A genes,
respectively, and without melancholia, showed a HAM-D decline under 17 compared to about 34% of the
whole study sample and the four other clusters. Our random forests prediction model for treatment
outcome showed that combining clinical and genetic variables gradually increased the prediction
performance recognizing correctly 25% of responders using all 4 factors. Conclusion: This is the first
study combining the analytic tools random forests and clustering in the investigation of MDD. Based on
the unique patient sample in TRD provided by the GSRD we could show the strength of data mining and
pattern analysis. Our findings substantiate the importance of interactions in complex neuropsychiatric
disease as MDD as we could confirm our previous findings and furthermore in identify new mechanisms
of treatment predictors.

242 Nature-derived circular peptides as oral treatment against experimental autoimmune
encephalomyelitis

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Multiple sclerosis is a chronic inflammatory disease of the central nervous system leading to
demyelination and neuronal degradation. There are a few therapy options available targeting different
aspects of this T-cell dependent autoimmune signalling cascade; most of them have to be administered
by injection, whereas those that can be taken orally induce many and severe side effects due to their
systemic mode-of-action. Consequently, there is still need for the development of new therapeutics. In
the past, naturally-occurring peptides have yielded a number of bioactive agents with promising
pharmaceutical properties. Therefore we analyzed the immunosuppressive properties of a unique class
of circular plant peptides, so-called cyclotides. Cyclotides are arranged in a typical cyclic cystine-knot
motif, which confers exceptional biophysical stability and makes them attractive pharmaceutical tools.
We have demonstrated concentration-dependent anti-proliferative effects of cyclotides towards
activated CD3+ lymphocytes in vitro, without inducing cytotoxicity. The down regulation of interleukin-2
cytokine release and CD25 surface expression at both protein and mRNA level further supported this
observation. Consequently, we investigated the activity of cyclotides as immunosuppressive therapeutics
using the state-of-the-art multiple sclerosis model experimental autoimmune encephalomyelitis.
Treatment of mice with cyclotides resulted in a significant delay and minor symptoms of the disease in
mice under prophylactic and therapeutic administration. Oral application not only substantially impeded
the progression of this autoimmune disease, but also improved the health status of the treated mice.
In summary, cyclotides are bioactive plant peptides with a unique structural topology. They appear to be
orally active towards treatment of experimental autoimmune encephalomyelitis and therefore may have
great potential as immunosuppressive peptide therapeutics.
Poster Session “Bones, Joints & Teeth”, 13.00 - 15.00
Chaired by Oskar Hoffmann

243 Potential mechanism for osseointegration of dental implants in ZDF rats
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Objectives: The aim of this project is to investigate the impact of diabetes mellitus and different glycemic control times on early osseointegration of dental implants, and to explore possible mechanism by expression and significance of integrin α5β1 and fibronectin (FN) in osseous tissue around implant.

Materials and Methods: 33 Male ZDF Rats 3 months old and weighing 450g at the beginning of the experiments were divided into three groups: Group A, diabetic rats with dental implants (controls); Group B, diabetic rats treated with insulin and implants placed simultaneously; Group C, diabetic rats treated with insulin until serum glucose at a constant level and then implants be placed. Rats were sacrificed at 7, 14, 30, 60 days after implant surgery in batches. Immunohistochemistry analysis was used to detect the expression of integrin α5β1 and FN in osseous tissue around the implants in each group.

Results: Fibronectin and integrin α5β1 were detected in osseous tissue around the implants. The expression of integrin α5β1 and FN in Group C were stronger than the other two groups (p<0.05). 14 days after implantation, expression of integrin α5β1 in group B was significantly stronger than that in group A (p<0.05). 60 days after implantation, the expression of FN in group B was significantly stronger than that in group A (p<0.05).

Conclusions: Both Fibronectin and integrin α5β1 participate in adhesion of osteoblasts and act as positive signal of bone implant interface. Diabetes interfere implant osseointegration by way of deferring expression of Fibronectin and integrin α5β1.

244 Bone density and bone metabolism in patients suffering from hepatic cirrhosis: Preliminary data
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245 Risk factors for deep periprosthetic joint infections after total hip arthroplasties
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The total hip arthroplasty (THA) is an effective operation for the restoration of the hip function. The number of operations is steadily climbing and is going to reach new heights in the future. The most devastating complication is the deep infection of the joint and has to be treated with a total revision of the prostheses. There are many different opinions on the risk factors in the literature. Our goal was to analyze and find the real risk factors, to minimize the infection incidence. We searched the database “PubMed” and “Embase” with the keywords: “(((hip AND infection)) AND (arthroplasty OR replacement))”. With the help of check lists and limits we extracted the most viable studies for our research. Risk factors associated with a deep infection included the BMI (Body mass index), male gender, prolonged duration of surgery, diabetes mellitus type 2, the ASA (American society of anesthesiologists) score, the Charlson score and the NNIS (National Nosocomial Infections Surveillance System) risk index score. Female gender, age and the diagnosis of rheumatoid arthritis were not associated with deep infections. Patients with risk factors should be assessed preoperatively and receive an appropriate prophylactic therapy. With the reduction of their weight and adjustment of the diabetes the patients can reduce the risk for infection by their own. Total hip replacement is still a safe and effective operation and thus should not be withheld from patients.

Scientific interests of 21st century clinical oral implants research: topical trend analysis

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6-Digit Implant Treatment Protocol Classification (ITPC): Application and Systematic Review of Scientific Literature


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In the last decade implant restoration has become a well accepted treatment option for edentulous jaw areas. Various classifications aiming to describe different treatment modalities in oral implantology are available in the current literature, each one of them considering only one specific step of implant therapy. The 6-digit system presented (ITPC) covers all aspects of in oral implant treatment. The aim of
this study was to provide a survey of treatment protocols available in dental implantology by the use of newly developed ITPC. Methods: Electronic as well as manual searches of English literature published from 2001-2012 were performed to identify clinical studies on dental implants, that yielded a total of 611 publications reporting on 67,106 implants. Results: ITPC as category wise, timing of implant placement in relation to tooth extraction were immediate (I) in 16.2%, early (E) in 1.6% and delayed (D) in 82.2%. Timing of bone grafting were no graft (N) in 63.6%, immediate graft (I) in 0.9%, pre-implant (P) in 19.4% and simultaneous graft (S) in 16.1%. Timing of soft tissue grafting were no graft (N) in 99.2%, immediate graft (I) in 0.1%, pre-implant graft (P) in 0.1%, simultaneous graft (S) 0.5%, staged graft (2) in 0.02% and delayed graft (D) in 0.003%. Healing modality were 1-stage (1) in 49.3% and 2-stage (2) in 50.7%. Timing of provisionalization were no provisionalization (N) in 59.5%, immediate (I) in 28.1%, early (E) 4.2% and 8.2% delayed (D). Timing of final prosthetic loading were immediate (I) in 5.3%, early (E) 7.0% and 87.7% delayed (D). Most frequently used ITPC in the literature were DNN2ND (17.7%), DNN1ID (15.2%), DPN2ND (14.3%), DSN2ND (7.1%) and INN1ID (7.1%). Conclusion: In the majority of cases implants are placed directly into alveolar ridge without performing bone augmentation and or soft tissue grafting. In recent years in the literature revealed a trend towards 1-stage healing modality and immediate provisionalization.

248 Dental pulp response to the prolyl hydroxylase inhibitor L-mimosine increases the production of Vascular Endothelial Growth Factor under diabetic conditions

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Prolyl hydroxylase (PHD) inhibitors can induce a pro-angiogenic response that stimulates regeneration of soft tissue in the dental pulp. A major problem of healing in diabetes is the high blood glucose level which leads to the formation of advanced glycosylation endproducts (AGEs) compromising regeneration. A novel strategy to stimulate the regenerative capacity are PHD inhibitors which target the cellular oxygen sensors. Thus the cell expression of vascular endothelial growth factor (VEGF) is enhanced, a key growth factor in angiogenesis and regeneration. However, it remains unclear if PHD inhibitors promote pulp regeneration under diabetic conditions. The impact of L-mimosine (L-MIM) on dental pulp regeneration was assessed in monolayer cell cultures and in tooth slice organ cultures in the presence of
AGEs to mimic diabetic conditions. VEGF, IL-6, and IL-8 production was measured by enzyme-linked immunosorbent assay (ELISA) to the pro-angiogenic and pro-inflammatory capacity of the dental pulp cells. Alkaline phosphatase staining was performed to discover the impact of L-MIM on the odontogenic differentiation. In addition histologies were performed from the tooth slices. We found that L-MIM enhanced VEGF and IL-8 expression in both, cell and tooth slice cultures, under diabetic conditions. IL-6 was not significant increased. Alkaline phosphatase staining revealed that odontogenic differentiation is dose-dependently reduced by L-MIM. Histological evaluation of tooth slices by MSB staining revealed no signs of necrosis or apoptosis upon AGE or L-MIM stimulation. Overall, the PHD inhibitor L-MIM enhances VEGF and IL-8 expression in both, monolayer cell cultures and the tooth slice organ culture model under diabetic conditions. L-MIM decreases the odontogenic differentiation dose-dependently in the dental pulp under diabetic conditions. This study serves as basis for upcoming in vivo studies to reveal the capacity of PHD inhibitors for dental pulp regeneration.

249 Mucin containing artificial saliva enhances the pro-inflammatory capacity of human gingival fibroblasts

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Saliva substitutes are frequently prescribed to patients due to the lack of saliva, so-called Xerostomia. Xerostomia results in a pro-inflammatory environment associated with impaired wound healing of oral tissue. To overcome the burden of Xerostomia manufacturers supplement their products with mucin, a large barrier forming glycoprotein, which physiologically resides in human saliva of healthy patients covering the oral surface to support lubrication in the digestion process. In addition, mucin triggers cell signaling in inflammation promoting cell expression of related factors. However, it is unclear if mucin-containing saliva substitutes modulate pro-inflammatory chemokine expression in human gingival fibroblasts. Mucin supplemented (Orthana AS® and Saliva natura®) and not supplemented (Aldiamed®, Glandosane®) saliva substitutes were evaluated regarding their pro-inflammatory impact on human gingival fibroblasts. Mucin1 (MUC1) from the bovine submaxillary gland and recombinant MUC1 were included in the bioassay. A panel of pro-inflammatory chemokines were measured by RT-PCR and ELISA. Viability was measured via formazan formation and LIVE/DEAD® staining. Signaling pathways were investigated with western blotting and pharmacological inhibitors. We report that Orthana AS® increases chemokine (C-X-C motif) ligand 8 (CXCL8 or interleukin 8), CXCL1 and CXCL2 expression on mRNA and protein level. The applied concentration of saliva substitutes did not reduce the viability. MUC1 isolated from bovine submaxillary glands and recombinant MUC1 significantly increased CXCL8, CXCL1 and CXCL2 chemokine expression in human gingival fibroblasts. MUC1 activates chemokine expression over NF-κB pathway. Inhibition of NF-κB with BAY 11-7082 blocked the chemokine expression of gingival fibroblasts.
significant. These results contributing to the role of glycoproteins in inflammatory gene expression of gingival fibroblasts.

250 Comparative analysis of the characteristics of fast-setting PMMA-cements

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This scientific work is a non-interventional, experimental and prospective comparative study of two very high-viscosity PMMA bone cements DePuy CMW 2G and Palacos fast R+G (Reference product: Palacos R+G). Fast-setting PMMA bone cements are used in the endoprothetics of the patella and knee (Australia) and to cement an artificial acetabulum (UK). All cements were mixed as specified by the manufacturer and the following parameters were analysed: handling properties (mixing, waiting, working and hardening phase), powder/liquid-ratio, mechanical properties (ISO 5833 and DIN 53435), fatigue strength (ISO 16402) and elution profile. All tests were done in an acclimatised laboratory at 23.5°C ± 0.5°C and >40% humidity. 11 units of 2 batch numbers of each bone cement were tested. The handling properties of the two tested PMMA bone cements Palacos fast R+G and CMW 2G are highly similar (n=12). Due to a higher powder/liquid-ratio of 2.550 of Palacos fast R+G, the cement has a shorter mixing, waiting and hardening phase than CMW 2G with a ratio of 2:1, therefore Palacos fast R+G is superior as it minimises the length of surgeries. Handling with Palacos fast R+G was advantageous due to its green dye. Mechanical properties according to ISO 5833:2002 and DIN 53435 were comparable. Palacos fast R+G had a statistically significantly higher ISO compressive strength (MPa, n=20, p≤0.01) at 0.05 level of significance. CMW 2G has a higher quasi-static ISO bending strength (MPa), but the same test shows a much higher fatigue strength (ISO 16402) of Palacos fast R+G (n=5). Palacos® fast R+G was far superior in matters of gentamicin release (n=3) over the time (24h, 72h and 120h), in particular due to its hydrophilic polymer basis. CMW 2G releases approx. only 1/3 of gentamicin per mould body after 24h. In conclusion, our results point to a bright future of fast-setting PMMA cements in the endoprothetic field and a superiority of the new Palacos fast R+G over CMW 2G.

251 Synovium-on-a-chip: Inflammatory tissue dynamics in arthritis regulated by endocytic membrane trafficking

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The synovium is a complex tissue primarily formed by fibroblast-like synoviocytes. The normal synovium demonstrates a lining/sublining architecture. During the course of joint inflammation, synovial tissue remodelling yields an aggressive cell mass that invades into and destroys the articular cartilage. Ultrastructurally, the synoviocytes display expansion of endocytic compartments. Based on these observations, we hypothesize that inflammatory cues impinge on endocytic trafficking pathways to bring about a deleterious synovial response. To gain insight into these processes, we have established a 3D synovial culture system on-a-chip. For this, synoviocytes were embedded in Matrigel and cultured for an extended period of time within the biochip. After 3 weeks, the cells established a lining/sublining cellular organization. Strikingly, when stimulated with the pro-inflammatory cytokine TNF, the synoviocytes reorganized and formed a condensed cell mass. Thus, the 3D synovial culture system on chip recapitulates characteristics of both, the normal as well as the diseased synovium. We will use gene silencing techniques, electron microscopy, life cell imaging, spectroscopy impedance measurements, and immunodetection of the secretome to examine how endocytic trafficking pathways control the inflammatory adaptive response of the tissue. These studies will open new avenues for the exploration of targets for the therapeutic intervention in patients with inflammatory joint diseases such as rheumatoid arthritis or osteoarthritis.

252 Glycine-based polyphosphazene scaffolds support chondrogenic differentiation of adipose derived stem cells


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Polyphosphazenes provide interesting inherent features for tissue engineering applications due to their highly diverse properties, depending on the side group substituents. The aim of this study was to evaluate the applicability of glycine-based polyphosphazenes as scaffolds for cartilage regeneration. Photo-polymerizizable scaffolds with controlled hydrolytic degradability were designed and prepared via short chain poly(organo)phosphazene building blocks. Porous matrices with or without glutathione were fabricated by thiol-ene photo-polymerization and subsequently seeded with adipose derived stem cells (ASC). Cell adhesion and proliferation were quantified and cells were then differentiated for up to 5 weeks under chondrogenic conditions. Micromass pellet cultures and TissuFleece®, a collagen sponge which recently demonstrated superior features to clinically applied biomaterials, served as controls. While cell adhesion and proliferation were slightly higher on TissuFleece®, qRT-PCR showed significantly higher collagen type II (COL2A1) and aggrecan (AGC1) expression for the glutathione containing
polyphosphazene compared to the controls. The expression of these key chondrogenic markers was slightly inferior for the polymer without glutathione, demonstrating expression in the range of TissuFleece®. Collagen type II immunohistochemistry and alcian blue staining corroborated the gene expression results. Quantification of glycosaminoglycans using dimethyl-methylene-blue demonstrated significantly enhanced GAG expression in glutathione containing polyphosphazenes compared to pellet cultures. In conclusion, glycine based polyphosphazenes demonstrated excellent properties for supporting synthesis of cartilage matrix proteins. ASC showed significantly higher chondrogenic potential when seeded to polyphosphazene polymers, especially at higher hydrophilicity in the presence of glutathione.
Total retinal blood flow measurement in the human eye with 3-Beam Doppler Optical Coherence Tomography

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Several eye diseases like age-related macular degeneration, diabetic retinopathy and glaucoma have been shown to be linked to alterations in the retinal blood circulation. Therefore quantification of the absolute retinal blood flow is of particular interest, but remains a challenge. To address this challenge we are using an improved 3-beam Doppler optical coherence tomography (DOCT) technique to measure the total retinal blood flow and obtain flow/velocity profiles within all major retinal vessels originating from the optic nerve head (ONH). The system consists of 3 independent SLD sources with a central wavelength of 840 nm. The collimated exiting beams share a common bulk optics Michelson interferometer. A well-defined beam geometry enables the full reconstruction of the three dimensional velocity vector, without prior knowledge on the vessel geometry, which is normally required for DOCT systems with less than 3 beams. A two axis gimbal less MEMS mirror allows raster, circular and resonant scan patterns, which are not practical with a classical 2 axis galvo scanner, because of heavy beam movement at the pupil of the eye, caused by off-pivot point scanning. Eyes of healthy subjects were imaged with a circular scan pattern and the mean total retinal blood flow as well as the velocity profiles inside all major retinal vessels emerging from the ONH were extracted and visualized. Furthermore 3 beam DOCT allows the reconstruction of the vessel geometry, showing excellent agreement between the actual and calculated vessel orientation as well as the flow direction. As an example, in a healthy eye, the total venous mean flow was measured to be 54.7 µl/min, while the total arterial flow was 47.8 µl/min. In conclusion the improved 3 beam DOCT technique allows the direct measurement of total retinal blood flow as well as
velocity vector determination with various scan patterns independent from any a-priory knowledge on the vessel geometry.

254 Assisted cardiac hemodynamics during exercise derived from left ventricular assist device signals

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Due to the lack of hemodynamic sensors, monitoring based on available pump data offers the only way to investigate the cardiac assisted hemodynamics. Here we report results of an ongoing clinical study about pump derived diagnostics in left ventricular assist device (LVAD) patients with the focus on exercising and physical capacity testing. To obtain pump data in patients a previously developed recorder device was used to store the data stream from a LVAD (HVAD, Heartware, Miami Lakes, FL) at a rate of 50Hz. Algorithms to estimate pump flow (Q) with increased frequency content, heart rate, and other derived indices of residual cardiac function were applied to the data. From 5 patients during cardiac rehabilitation a total of 24 bicycle ergometry, 19 walking, 11 leg strength and 17 gymnastic training sessions were analyzed. Furthermore physical capacity testing with ergospirometry (n=2), stress-echocardiography (n=3) and 6 minute walking test (6-MWT, n=5) was investigated. Hemodynamic parameters derived from the LVAD were combined with clinical relevant diagnostics and exercise specific documentation during training and physical capacity testing. Amongst training, interval bicycle training triggered the highest response of cardiac function as derived from the pump data. During bicycle training an increase in heart rate (5±1 bpm), mean pump flow (0.45±0.2 L/min) and pulsatility (0.5±0.2 L/min), as well as cardiac function indices with respect to baseline were observed (p ≤ 0.0004 in all cases). Ergospirometry and stress-echocardiography lead to even greater increases in heartrate (17±12 bpm), pump flow (1.5±0.9 L/min) and pulsatility (1±1.5 L/min). Cardiac adaption occurred also during the submaximal physical capacity testing with the 6-MWT with an increase in heartrate (11.8±6.5 bpm), pump flow (1.1±0.7 L/min) and pulsatility (0.9±0.7 L/min). This ongoing study demonstrates that continuous LVAD-monitoring during exercise and physic

255 Electrospinning small diameter vascular grafts with tunable fiber orientations

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Aim: The need for surgical revascularization therapies in small diameter applications is constantly increasing. Autologous vessels are gold standard but not always available. Vascular grafts which are comparable with those are essential. Electrospinning offers fabrication of fibrous scaffolds imitating the extracellular matrix. During conventional electrospinning fibers are deposited in a chaotic fashion due to various instabilities. Increased control of fiber deposition is essential to manufacture grafts which can mimic the complex layered structure and the biomechanical behavior of the host vessel. Methods: Polymeric tubular vascular grafts were electrospun from Pellethane® 2363 80A on metal mandrels with a diameter of 2mm. Orientation and fiber alignment was controlled by auxiliary plate-like electrodes using electrodynamic deflection of the electrospinning jet. Prostheses with random, circumferential, longitudinal and 30° fiber direction were fabricated. Grafts were characterized by measuring the wall thickness and gravimetric porosity. Effects of fiber orientation were analyzed in the scanning electron microscope and by measuring the compliance in the physiologic blood pressure range. Results: The electrospun vascular grafts had a mean wall thickness of 71 ± 6µm. Lowest porosity of 63% was seen in circumferentially electrospun grafts whereas grafts spun with fiber directions in ± 30° showed the highest porosity of 79%. Fiber alignment in the main direction of each selected orientation angle was observed. The prostheses with longitudinal fiber orientation showed a compliance of 18.6 ± 2.8 %/100mmHg, whereas the prostheses with circumferential orientation exhibited the lowest compliance of 7.1 ± 2.6 %/100mmHg. Conclusion: The developed electrodynamic control method allows to electrospin small diameter vascular prostheses with pre-defined fiber orientations.

256 In-Vitro Measurement of Cannula Centering Forces for Transvalvular Ventricular Assist Devices

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Aim: Ventricular Assist Devices (VAD) are an established therapeutic option to provide hemodynamic support in patients suffering from end stage heart failure. In transvalvular VADs the outflow cannula is positioned across the aortic valve. To avoid backflow and thrombus formation, centering of the cannula within the valve orifice is essential. In this work influencing parameters on the radial forces on transaortic cannulas were investigated in a static and pulsatile in-vitro setup. Methods: Porcine aortic valves (n=3) were harvested from the abattoir and mounted in a test rig. On a linear motor (Bose® Electroforce LM1), equipped with a 10 N force transducer, three different cannula dummies (diameter: 4, 6 and 8 mm) were mounted. By the linear motor the cannula was deflected from the central position in 1 mm steps and the valve rotated in 30° steps. The centering forces exerted on the cannula were
measured in all positions at static and pulsatile transvalvular pressures from 60 to 100 mmHg. Results: Highest centering forces up to 1.12 N were found when the cannula was close to the aortic wall. Minimal forces were observed not just in a single point, but an area of low force was measured. The transvalvular pressure caused a linear increase in centering forces (+21±4% per 20 mmHg) in the static and pulsatile setup. In the static measurements slightly differing centering force patterns along the commisures and leaflets were observed, although single leaflets were not represented by the force distribution. Also the size of the cusp was not associated with the centering force. The diameter of the cannula had minor influence on the force and comparable force distributions were seen between all three cannula sizes. Conclusion: The centering force of the cannula is mainly effected by magnitude and dynamics of transvalvular pressure. The diameter of the cannula has a minor influence. A certain centering force is preserved also at low pressures.

257 Measurement of hepatic lipid content and composition in humans with proton ultra-short TE MR spectroscopy at 7T

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Ultra-high field whole-body MR systems increase signal-to-noise ratio (SNR) and improve spectral resolution. Sequences with short time-to-echo (TE) allow fast signal acquisition with low signal loss due to the spin-spin relaxation. This is of particular importance in the liver for precise quantification and profiling of hepatocellular lipids (HCL). In this study, we modified STEAM sequence into spoiler Gradient switching Ultra-short STimulated Echo AcQuisition (GUSTEAU) scheme with a minimum TE of 6 ms. With high spectral resolution, efficient elimination of water sidebands and post-processing suppression of water signal, we could estimate the composition of fatty acids (FA) via the detection of the olefinic lipid resonance and calculate the unsaturation index (UI) of hepatic FA. The performance of the GUSTEAU sequence regarding the UI assessment was validated on oil samples and provided excellent results in agreement with literature data. The comparison of the HCL in ten healthy volunteers measured with
GUSTEAU at 7T with 3T data showed high correlation ($R^2=0.961$) and the test-retest measurements yielded low coefficients of variation for HCL (4±3 %) and UI (11±8 %) measured with the GUSTEAU sequence at 7T. Negative correlation was found between the UI and the HCL ($n=10; p < 0.033$). Ultra-short time-to-echo MRS sequence (GUSTEAU; TE=6 ms) provided high repeatability for assessment of HCL. Furthermore improved spectral resolution at 7T with water sidebands elimination and offline water subtraction allowed assessing the unsaturation of FA. This all highlights the potential of this MRS acquisition scheme for studies of hepatic lipid composition in vivo.

258 Test-retest variability of human brain gray matter voxel-based morphometry at ultra-high fields of 7 Tesla

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Voxel-based morphometry (VBM) is a regularly applied method to assess gray matter changes due to disease progression or cognitive interventions in vivo. Routinely, images used for analysis are recorded at a field strength of 3 Tesla. However, recent technological progress enables MRI recordings at ultra-high fields of 7 T and above, leading to brain images of higher resolution and increased signal-to-noise ratio. Despite these benefits, imaging at higher fields exhibits distinct challenges, causing decreased image quality and problems in data analysis. Hence, it still remains a matter of debate if VBM analyses can be carried out properly in all areas of the brain at ultra-high fields. To assess the reliability of 7 T VBM measurements, results were compared to 3 T standard recordings by conducting a reliability analysis using test-retest variability (TRV). More specifically, ten subjects (mean age±SD=26.36±7.3, 6 females) underwent MRI measurements at two time points and were measured under the conditions 3T MPRAGE, 7T MPRAGE and 7T MP2RAGE. The TRV values varied strongly between the conditions 3T MPRAGE, 7T MPRAGE and 7T MP2RAGE. 3T MPRAGE measurements showed best overall variability results (in percent) across all regions of interest (1.6 ± 0.8), followed by 7T MPRAGE (4.5 ± 1.6) and 7T MP2RAGE (5.5 ± 4.6). However, analysis revealed that the TRV differed strongly amongst brain regions. While 3T MPRAGE data showed good reliability across the entire brain, 7T MPRAGE and 7T MP2RAGE indicated less reliability in several subcortical (thalamus, pallidum) and inferior regions (temporal gyrus, cerebellum, middle and inferior occipital gyrus) of the brain. Results suggest that VBM analysis, especially in subcortical regions as well as in inferior parts of the brain, should be done with caution. Substantial imaging artefacts in these areas were observed, causing problems in automated data analysis. However, in superior cortical regions stable results were achieved.

259 Eyetracker-based gaze correction for robust mapping of population receptive fields

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The neural population of a small area in the human primary visual cortex corresponds to a specific position on the retina, which itself only detects signals from a specific visual field region. Such a region is also being referred to as "population receptive field (pRF)". Functional magnetic resonance imaging (fMRI) allows for the acquisition of a so called "retinotopic map" of the visual cortex by mapping every measured voxel to its individual pRF. During a retinotopy experiment, stable gaze fixation of a predefined location is of utmost importance. Here we try to account for unstable fixation during fMRI by recording gaze position via an eyetracker and subsequently modifying the stimulus underlying the pRF analysis according to the eyetracker recordings. We measured six healthy subjects (4 female, age: 24 ± 4.2) with normal visual acuity on a 3T Siemens Trio scanner. FMRI images (TE/TR=30/1000 ms) were acquired with an effective voxel size of 1 x 1 x 1 mm³. The visual stimulus consisted of a moving bar, exposing a flickering checkerboard and crossing the screen in eight different directions. The subjects were instructed to fixate a small dot at the center of the screen. Aside from remaining stationary (static fixation), we also performed measurements where the dot changed its position randomly every 4 seconds (random fixation). During each measurement we acquired gaze position data using an EyeLink 1000 Plus (SR-Research, Ottawa, ON) eyetracker. Resulting pRF maps of the stimulus with the random fixation dot showed a strong improvement of the mapping, as the agreement of static fixation and random fixation activation maps is much higher after performing the correction. Our results demonstrate that for unstable fixation, eyetracker-based pRF correction can be used to obtain high-quality mappings, which could help when dealing with patients who struggle with stable fixation, e.g. due to central scotomas, as in age-related macular degeneration.

260 Mobile gait analysis via instrumented shoe insoles - extraction of gait parameters and validation

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Personal mobility is a key factor for the preservation of a livable and self-determined lifestyle in old age, where several chronic diseases are occurring more frequently. Many chronic conditions are treated in stationary care and rehabilitation, where patients receive extensive therapy and physical training. After discharge patients often discontinue their training due to lack of motivation and supervision. Mostly
measurement equipment for rehabilitation support is expensive and cumbersome. To cope with this problem, a mobile, shoe insole based, motion analysis and feedback system eSHOE has been developed. eSHOE consists of a pair of orthopedic insoles, instrumented with motion and pressure sensors and data processing electronics. It collects data directly on the feet during mobility assessments and stores it on a microSD card or transmits them to a PC via Bluetooth. Standard gait parameters are extracted from the sensors’ raw data via special signal processing algorithms. The applicability and validity were tested in the course of a pilot study at a geriatric hospital with hip fracture patients (n=10) and healthy subjects (n=12). Validity was examined by measuring with eSHOE and GaitRite®, as reference system, in parallel during straight walking. Nine gait parameters have been evaluated and compared via scatterplots, histograms of the differences and Bland-Altman analysis. Evaluation of the comparison shows a high correlation of all parameters (r=0.84), narrow distribution of the differences between the two systems (mean difference: -0.006 sec) as well as satisfying values for the limits of agreement (0.078 to -0.091 sec). The results prove eSHOE to be valuable supplement to intramural gait analysis. In the following, remaining data about the progress of the rehabilitation will be analyzed. Current work focuses on the expansion into a rehab@home system, with a user interface for patients as well as remote access for medical experts via a web-interface.

261 Development Of At Home Rehabilitation And Prevention Procedures

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Rehabilitation is an indispensable component of recovery. After discharge from a rehabilitation clinic the recovery process is usually not fully completed. Rehabilitation at home after clinical care is becoming a key factor in the physical regeneration of patients. Due to demographic development the costs for rehabilitation are increasing. Telerehabilitation and rehabilitation at home may reduce such costs. Within the research project REHABitation, concepts are being developed for at home rehabilitation and prevention procedures, based on the validation of biomechanical motions and postural control. The focus is on the validation of biomechanical motions and postural control. Therefore a close cooperation with stakeholders and users is crucial.

In a preliminary study the eSHOE-system was developed as a monitoring tool for rehabilitation in clinical and home environments. The first investigations showed in a 10m walking test a mean cycle length of 1.051 ± 0.075s for healthy elderly subjects (n=12) and 1.367 ± 0.183s for geriatric patients (n=11). In addition, the practicability of the eSHOE-system after and during clinical care was tested in a pilot study with stroke patients (n=30). The results suggest that the eSHOE-system is suitable for the analysis of physiological and pathological movements. In the REHABitation project the eSHOE-system is used in combination with other devices as a modular system for Telerehabilitation.

262 The benefit of continuous flow left ventricular assist devices in NYHA III patients during rest and exercise. A computer-simulation study.

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Aim: Currently, left ventricular assist devices (LVAD) are exclusively indicated for NYHA IV patients. However, implantation of the device in earlier stages of heart failure is associated with better outcome. The purpose of the presented study is the investigation of the hemodynamic influence of a LVAD on less sick patients.

Methods: Published data on hemodynamic parameters were used to establish and validate a numerical model during rest and exercise. A first implementation and test was done without cardiac assistance. Furthermore, a previously validated LVAD model was added to calculate the changes in patient hemodynamics and performance with focus on exercise-related changes.

Results: The addition of a LVAD reduced left atrial pressure, thus reducing the associated dyspnoe. This enabled higher aerobic exercise capacity. While unloading of the ventricle was improved, the increase in cardiac output was only up to 25%, which would not be sufficient to compare to exercise tolerance of healthy subjects. Conclusion: LVADs do improve the hemodynamics in rest and especially in exercise, potentially allowing the patients for an increased physical capacity. However, with the currently available devices with limited flow below 8L/min, the increase does not sufficiently compensate for the required additional flow. Therefore, at the current stage of devices and adverse event probabilities the achievable benefit would probably not compensate for the risks of the implantation and the associated long term complications.

263 Influence of fovea position and retinal vessels in interindividual variability of healthy circumpapillary retinal nerve fiber layer measurements


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Purpose: To assess whether intersubject variability in healthy circumpapillary retinal nerve fiber layer (RNFL) measurements acquired with spectral domain optical coherence tomography (SD-OCT) may be reduced by considering the fovea angle (FA), either alone or together with a compensation based on the retinal vessel distribution (RVD). Methods: 106 healthy volunteers underwent complete ophthalmological examination including SD-OCT. For each subject, both SLO images centered in the optic disc (OD) and in the macula were acquired with SD-OCT. A proprietary software was developed in Matlab to manually assess OD contour, RVD at 3.4 mm diameter circle and fovea position. RVD is a function of retinal vessel thickness and its relative position to the OD. Both SLO images were manually registered and the angle between a line connecting fovea and OD centers and the horizontal axis was calculated.
Compensation for interindividual variability in RNFL was based in: 1st Compensation: RNFL thickness compensation by RVD; 2nd Rotation: RNFL measurements shifting by FA. The mean reduction of coefficient of variance (CoV) was calculated in 12 clock hour sectors and compared using paired t-tests for original, rotated and compensated RNFL (RNFLo, RNFLr and RNFLc, respectively), and both rotated and compensated RNFL (RNFLrc). Results: Compared to the mean CoV of RNFLo, the mean CoV of RNFLr, RNFLc and RNFLrc was changed by -0.47% (18.00% to 17.95%, p=0.905), -9.51% (18.00% to 16.26%, p<0.05) and -7.45% (18.00% to 16.66%, p<0.05), respectively. Compared to RNFLr, RNFLrc did significantly reduce the mean CoV (17.95% to 16.66%, p<0.05). Compared to RNFLc, RNFLrc did not significantly change the mean CoV (16.26% to 16.66%, p=0.782). Conclusions: Although improving in some sectors, rotation of RNFL measurements according to the FA, both original and compensated, do not present, on average, a reduction in intersubject variability of RNFL, as opposed to RVD compensation.

Photoacoustic Elastography with Texture Generation

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Elastography is a coupled imaging technique that maps the elastic properties of tissue. This is achieved by applying a mechanical force and measuring the resulting displacement, using e.g. ultrasound, optical coherence tomography or magnetic resonance imaging. Photoacoustic has not been used as a primary means to measure this displacement, but only as a complementary method to enhance contrast in ultrasound elastography. Photoacoustic generally is considered speckle free, which makes it seem less suited for Elastography. However, while conventional ultrasound only uses a single frequency, photoacoustic produces a broad frequency spectrum. We are therefore able to generate artificial texture by using a frequency band limited part of the recorded data. In this work we laterally apply a 50[µm] static compression to a phantom with predefined Young’s moduli. Pre- and post compression data is recorded via a Fabry-Perot interferometer planar sensor setup and reconstructed via a non-uniform-FFT reconstruction algorithm. A displacement vector field, between pre- and post compressed data is then determined via optical flow algorithms. The minimum displacement resolution turns out to be well below the opto-acoustical resolution of the system and reaches up to few µm. The speckle free nature of photoacoustic and its adverse effect on the application on photoacoustic elastography can be ameliorated by generating artificial texture, which makes the optical flow algorithm more reliable and reduces the variance of the measurements.

A Mechanical Eye Model to Evaluate the Quality of Vision and the Optical Performance of IOLs
Cataract surgery is one of the most frequent medical interventions with more than 11 million surgeries per year. To restore vision of the patient the clouded natural lens is extracted and replaced by an artificial intraocular lens (IOL). IOLs with different designs as well as optical properties such as monofocal, bifocal or multifocal lenses are used for implantation. Increased sophistication of intraocular lenses, leads to the fact that the impact of the implant’s position within the eye has to be treated more critical. To evaluate lens misalignments occurring due to postoperative shifts and tilts a closer investigation of the positioning sensitivity is necessary, to draw conclusions about the patient’s refractive outcome after treatment. A mechanical eye model with a tilt shift unit was implemented for testing different types of IOLs under physiological conditions. New measurement setups are presented to get more detailed information about optical quality criteria such as the modulation transfer function (MTF), point spread function (PSF) and Strehl ratio. Additionally image quality is evaluated by the determination of the Zernike coefficients to identify typical aberrations of the human eye. A projection mechanism allows the transfer of an image to the patients’ eye to judge the physiological impression of vision.

266 Determining the optimal flip angles for R1 maps using the variable flip angle (VFA) method at 7T

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The variable flip angle (VFA) method is a quantitative MR imaging principle, which exploits the flip angle dependence of spoiled gradientecho MRI to calculate R1 relaxation rates \( R_1 = 1/T_1 \) of tissue. This parameter can be estimated by fitting the data points to a linearized version of the Ernst equation. Optimal flip angles can be determined a priori to maximize the precision of the parameter estimate. Such an approach is insufficient at ultrahigh field (≥ 7T) where the transmit B1 field, and by extension the flip angle, is highly inhomogeneous. Here we extend the optimization procedure presented previously to account for B1 inhomogeneity during flip angle optimization.

In the previous implementation, the selection of flip angles was based on the simple minimum of the noise propagation function. To counter the problem of flip angle variability at 7T, we propose to integrate the noise propagation function over the curve described by the tangent half angle transformation of the flip angles weighted by the probability density function of the B1 bias distribution.
across the region of interest. The minimum of this function will then give the optimal flip angles for the given \( B_1 \) distributions.

To implement this method, we acquired \( B_1 \) maps from two subjects and empirically derived the probability density function in the brain for a range of physiological target \( T_1 \) values. The optimization procedure was experimentally tested by acquiring data from a third subject using the average set of optimal flip angles for the target \( T_1 \) range.

Using the new optimization scheme, we were able to calculate optimal flip angles that minimize noise propagation into \( R_1 \) maps at 7T.

This method enables reliable quantitative maps to be computed at high field strengths while maintaining whole brain coverage.
Signaling mechanisms of 5-HT2 receptors in primary sensory neurons

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Serotonin (5-HT) is involved in pain sensation. 5-HT3 receptors (5-HT3R) of dorsal root ganglion neurons (DRGs) are thought to mediate this effect. However, hyperexcitability of DRGs in the presence of 5-HT was not altered by the 5-HT3R antagonist tropisetron, but reduced by the 5-HT2 receptor (5-HT2R) antagonist ritanserin; the 5-HT2R agonist (±)-2,5-dimethoxy-4-iodoamphetamine (DOI) increased neuronal excitability. Since Kv7 and TRPV1 channels are important regulators of DRG excitability and pain sensation, the contribution of 5-HT2R and their functional interactions with Kv7 and TRPV1 channels were investigated in DRGs electrophysiologically. Kv7 channel currents of DRGs were unaffected by 5-HT, but reduced by DOI in a concentration-dependent manner. Additionally, Kv7 channels were inhibited by ritanserin, and the effects of DOI and ritanserin were additive. Currents through Kv7.2/7.3 heteromers expressed in tsA201 cells (in absence of 5-HT2Rs) were also significantly attenuated by DOI and ritanserin, but were altered neither by the 5-HT2R antagonist ketanserin nor by 5-HT. In tsA201 cells coexpressing 5-HT2R and Kv7.2/7.3 heteromers, 5-HT failed to suppress Kv7 currents. Recombinant 5-HT2ARs and 5-HT2CRs, nevertheless, mediated increases in intracellular Ca2+. In DRGs, 5-HT2R activation enhanced TRPV1 currents. The enhancement was inhibited by ritanserin, ketanserin, 5-HT2AR antagonist 4F4PP oxalate and 5-HT2CR antagonist RS102221 hydrochloride. Moreover, the enhancement of TRPV1 currents by 5-HT was prevented by the inhibition of PLC, Ca2+-ATPase, PKC and by Ca2+ chelation. In contrast, 5-HT-induced DRG hyperexcitability was inhibited by blocking TRPV1s. These results indicate that DOI and ritanserin can interact directly with Kv7 channels. In DRGs, activation of 5-HT2R mediates enhanced excitability, an effect that involves sensitization of TRPV1 channels. Thus, the potentiation of TRPV1 channels by 5-HT involves PLC, intracellular Ca2+ and PKC.

Glial-neuronal interactions in nociceptive transmission

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Amplification of synaptic strength at the first synapse in a nociceptive pathway is a cellular model for enhanced pain sensitivity. Recent evidence suggests that solely factoring neuronal activity in terms of synaptic plasticity provides an incomplete understanding of the establishment of amplified nociceptive transmission. Although glial cells have emerged as key modulators of synaptic plasticity, so far it has not been shown whether their activation alone is sufficient to amplify synaptic strength. We used 2′(3′)-O-(4- Benzoylebenzoyl)adenosine 5′-triphosphate triethylammonium salt (BzATP)-induced P2X<sub>7</sub> signalling to activate glial cells in the dorsal horn and studied the effect on synaptic transmission between nociceptive C-fibres and lamina I neurons in an electrophysiological approach. Surprisingly, application of P2X<sub>7</sub> receptor agonist BzATP induced a significant depression of synaptic transmission. We could show that this BzATP-induced depression was not mediated by P2X<sub>7</sub> signalling but by adenosine acting on inhibitory A<sub>1</sub> receptors. Activation of glial P2X<sub>7</sub> receptors under blockade of A<sub>1</sub> receptor signalling induced a long-term potentiation (LTP) in 64% of all neurons tested. The BzATP-induced potentiation was accompanied by a significant reduction of the paired pulse ratio (PPR), suggestive of a presynaptic expression of LTP. This decrease of PPR could neither be observed in neurons that did not show a response to BzATP application nor under blockade of P2X<sub>7</sub> receptors prior to BzATP application. Blockade of P2X<sub>7</sub> receptor signalling by the specific antagonist A-438079 completely prevented the BzATP-induced potentiation, whereas blockade of A<sub>1</sub> receptor signalling alone had no significant effect on synaptic transmission. Here, we could show for the very first time that activation of glial cells is sufficient to significantly amplify synaptic transmission at the first synapse in nociceptive pathways.

19 Unexpected effects of selected cytokines on osteoclast generation


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Background and Aim: The proinflammatory cytokines interleukin-1 beta (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) traditionally have been regarded as bone resorptive cytokines. Nevertheless, a few recent publications give evidence for inhibitory effects of TNF-α on osteoclastogenesis. The aim of this work was to analyze the effects of IL-1β, IL-6 and TNF-α on osteoclastogenesis and the expression of cathepsin K, an enzyme essential for bone resorption, in primary murine bone marrow osteoclast cultures. Methods: Primary murine bone marrow cell cultures from HIM:OF-1 mice were supplemented with 1,25 dihydroxy vitamin D3 to induce osteoclastogenesis and treated with IL-1β, IL-6 and TNF-α. After a culture period of one week, cells were stained for tartrate resistant acid phosphatase (TRAP). Furthermore, mRNA levels of cathepsin K and of the major regulator of osteoclast generation, receptor activator of NF-κB ligand (RANKL) and its decoy receptor osteoprotegerin (OPG) were determined by real-time-PCR. Protein expression of cathepsin K was assessed by immunofluorescence staining. Results: Combined treatment and treatment with the individual cytokines significantly decreased the number of generated osteoclasts as assessed by TRAP.
staining (combined treatment: -63.3%, IL-1β: -39.1%, IL-6: -37.1%, TNF-α: -52.39%), but had no effect on protein expression and mRNA expression of cathepsin K. However, there was a trend towards a lower cathepsin K mRNA expression in cultures individually treated with interleukin-6 or TNF-α and a lower RANKL/OPG mRNA ratio in cultures individually treated with TNF-α. Conclusion: We conclude, that in our experimental setting the proinflammatory cytokines IL-1β, IL-6 and TNF-α decrease the generation of osteoclasts, but do not effect mRNA or protein expression of cathepsin K.

20 Anterior eye and retinal imaging in mice using high resolution polarization sensitive OCT


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A vast variety of disease models have been developed in mice, and they are widely used in basic research. For instance models of sight threatening disease such as age-related macular degeneration (AMD) were introduced. Since the pathophysiology of this disease is not yet fully understood, animal models need to be closely studied longitudinally. In longitudinal studies, non-invasive diagnostic methods providing enough information are desired. For the eye, optical coherence tomography (OCT) is one of the best options. In this study, we have developed a high resolution polarization sensitive OCT (HR-PS-OCT) system for imaging small animals, including mice. By extending standard OCT by polarization sensitive detection we can gain more information from the sample. Together with morphological information we can acquire images showing depolarization and birefringence. This can be used e.g. for segmentation of pigmented tissues (pigmented tissues show depolarization) and sclera (shows birefringence). Since pigmented layers, such as choroid and retinal pigment epithelium (RPE), are affected in AMD, HR-PS-OCT can be used to study these in animal models of this disease. The axial resolution of the system is 3.8 μm in tissue. Using custom built spectrometers, a 3D dataset is acquired in less than 4 s. The short scan time enabled by the high imaging speed reduces motion artefacts in volumetric images. In this study, anterior eye and retina were imaged in C57BL/6 mice. In the anterior eye, pigmentation of the iris was detected by depolarization analysis in PS-OCT images. In the retina, all layers were identified on morphological images. Pigmentation in RPE-choroid complex was detected. The choroid is more densely pigmented than RPE, as was also observed in Long-Evans and Brown-Norway rats. Since the system provides high-resolution morphological images and visualization of pigmented tissues, it may be a valuable tool for in-vivo imaging of AMD mice models.