1 Showing Strength: Mechanical Forces in T-Cell Antigen Recognition

Kellner F (1), Göhring J (1,2), Schrangl L (2), Platzer R (1), Schütz GJ (2), Huppa JB (1)
(1) Institute for Hygiene and Applied Immunology, Medical University of Vienna, (2) Institute of Applied Physics, Vienna University of Technology
florian.kellner@meduniwien.ac.at

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 calibrated 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. 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 of pMHCs and simultaneously imaged downstream signaling. In summary, we expect to visualize TCR-imposed forces most directly and establish them as a crucial factor in T-cell antigen recognition.

2 mTORC2 regulates macrophage polarization and the cellular energy metabolism

Katholnig, K., Hirtl, M., Linke, M., Sukhbaatar, N., Herac, M., Spittler, A., Hengstschläger, M., Weichhart, T.
Institute of Medical Genetics, Medical University of Vienna
karl.katholnig@meduniwien.ac.at

Macrophages are innate immune cells and pivotal for the regulation of immune and metabolic responses. A dysregulation of immune or metabolic processes contributes to 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. 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 in vitro. Rictor deletion 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. In line, less macrophages accumulate in adipose tissue of macrophage-specific Rictor-KO mice on high-fat diet compared to control mice. Molecularly, glucose influx and mitochondrial membrane potential are reduced in Rictor-deficient macrophages. These defects in the cellular energy

metabolism contribute to the reduced M2 polarization and proliferation in these cells, as the use of the glycolytic inhibitor 2-DG in WT macrophages phenocopies the effects of the Rictor-KO macrophages. Interestingly, we can link the observed stronger in vitro inflammatory phenotype of Rictor-KO macrophages to a worsened response in an acute DSS-colitis model in vivo. In this model, loss of mTORC2 in macrophages leads to increased weight loss and decreased survival of the mice. In conclusion, our results point to an important role of mTORC2 in the regulation of macrophage polarization, cell cycle progression and migration that contributes to the onset of severe inflammatory diseases, such as colitis.

3 Live fast – die young: Urokinase receptor expressing T cells are adherent, hyper-reactive and undergo activation induced cell death

Zwirzitz, A. (1), Leksa, V. (2), Elbe-Bürger, A. (3)
(1) Medical University of Vienna, Center for Pathophysiology, Infectiology & Institute for Hygiene & Applied Immunology, (2) Laboratory of Molecular Immunology, Institute of Molecular Biology, Academy of Sciences, Bratislava, Slovak Republic, (3) Division of Immunology, Allergy and Infectious Diseases, Department of Dermatology, Medical University of Vienna
alexander.zwirzitz@meduniwien.ac.at

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.

4 An anti-inflammatory role for Alk3 in Langerhans Cells

Hochgerner, M. (1), Borek, I. (2), Bauer, T. (1), Sibilia, M. (1), Strobl, H. (2)
(1) Institute of Cancer Research, Medical University of Vienna, (2) Institute of Pathophysiology and Immunology, Medical University of Graz
mathias.hochgerner@meduniwien.ac.at

Langerhans Cells (LCs) represent the epidermal contingent of dendritic cells. They are thought to perform two seemingly contrasting functions as they can act both pro- and anti-inflammatory. Formation and maintenance of the epidermal LC network is dependent on the cytokine TGF-beta1. Genetic disruption of TGF-beta1 signaling in LCs results in the activation of LCs leading to their egress to skin draining lymph nodes shortly after birth. Using in vitro differentiation models, we previously demonstrated that human LC differentiation can be induced by bone morphogenetic protein (BMP)-7, another member of the TGF-b superfamily of related molecules. Our current hypothesis is that Alk3, a Type I receptor induced by both BMP-7 and TGF-beta, is necessary for LC differentiation and proliferation but is insufficient for anti-inflammatory function. Conversely, ALK5 activated by TGF-beta1 but not BMP-7 is critical for LC network maintenance. Congruent with this hypothesis, we recently observed strong upregulation of BMP-7 as well as phosphorylation of its downstream components SMAD1/5/8 in inflamed human psoriatic epidermal keratinocytes. We generated a number of conditional knock-out mice to test this hypothesis in vivo. Experiments with Alk3-flox CD11c-cre mice showed that knock-out mice display normal amounts of LCs in steady-state skin; no adverse effects could be observed. Conversely, when subjected to Imiquimod-induced skin inflammation (a model of psoriasis), knock-out mice displayed a stronger and longer lasting inflammation. Moreover, a migration assay showed that LCs from knock-out mice migrate faster than from wildtype mice with emigrated LCs showing a trend to a higher expression of MHCII and CD86. Therefore, BMP-7- Alk3 signaling is upregulated in psoriasis and may counteract inflammation via an LC-mediated mechanism.

5 The role of STAT3 in polarization of tumor-associated macrophages

Pflügler S. (1), Svinka J.(1), Pathria P.(2), Knapp N.(3), Strobl B.(4), Müller M.(4), Eferl R.(1)
(1) Institute of Cancer Research, Medical University of Vienna, Austria. (2) Moffitt Cancer Center & Research Institute, Florida, USA. (3) Boehringer Ingelheim RCV GmbH & Co KG, Vienna, Austria. (4) Institute of Animal Breeding and Genetics, University of Veterinary Medicine Vienna, Austria.
sandra.pfluegler@meduniwien.ac.at

Activation of the transcription factor STAT3 in tumor and immune cells is important for immune escape of many human cancers. We demonstrated that formation of azoxymethane/dextransulfate induced colorectal cancers was strongly suppressed in mice lacking STAT3 in myeloid cells/macrophages. Gene expression profiling showed that STAT3-deficient macrophages were M1 polarized and triggered strong anti-tumor T cell responses in the tumor stroma. Macrophage polarization is influenced by TLR signaling, indicating that the intestinal microbiome influences anti-tumor immune responses in colon cancer. We used in vitro macrophage cultures to gain insight into tumor – stroma interactions modulated by STAT3 activation in myeloid cells. As a cellular source, we isolated bone marrow cells from mice with conditional deletion of STAT3 in the myeloid compartment and differentiated them in vitro into mature F4/80[up+] CD11b[up+] macrophages. The macrophages were then stimulated with TLR ligands to assess STAT3-dependent functions in macrophage polarization. We demonstrate that STAT3-deficient macrophages are strongly activated by TLR4, TLR2/6 and TLR7/8 ligands, but did not differentially respond to TLR5 stimulation when compared with control macrophages.

Differential activation of STAT3-deficient macrophages was also reflected by increased expression of M1 polarization markers and altered phagocytosis. STAT3 inhibitors are considered for treatment of colorectal cancer. Our data suggest that the presence of certain TLR ligands, present within the intestinal microbiome, might influence pharmacological effects on macrophage polarization and therapeutic response.

6 Impact of structural modifications of novel thiosemicarbazones on the mechanism and strength of their antineoplastic activity

Hager, S. (1,3), Miklos, W. (1,2), Pfaff, S. (2,3), Enyedy, É. A. (4), Kowol, C. R. (2,3), Keppler, B. K. (2,3), Berger, W. (1,2), Heffeter, P. (1,2)
(1) Institute of Cancer Research and Comprehensive Cancer Center, Department of Medicine I, Medical University Vienna, Austria (2) Research Platform, Translational Cancer Therapy Research Vienna, Austria (3) Institute of Inorganic Chemistry, University of Vienna, Austria (4) Department of Inorganic and Analytical Chemistry, University of Szeged, Hungary
sonja.hager@gmx.at

Thiosemicarbazones containing an alpha-N-heterocyclic moiety are well known for their antineoplastic activity as well as their ability to form complexes with many transition metals. One of them, Triapine, is being extensively studied in clinical trials for cancer therapy with promising activity against advanced leukemia. The aim of this study was to investigate the effect of structural modifications of Triapine on the mechanism and strength of their anticancer activity. Stepwise methylation of both amino groups of Triapine resulted in eight novel derivatives. The compound with the highest methylation grade (Me[down2]NNMe[down2]) showed a 17-fold higher anticancer activity in the nanomolar range in SW480 cells. In contrast, only a small increase or even decrease in cytotoxicity compared to Triapine was found for all other derivatives with lower methylation grade. Further examinations on cytotoxicity in combination with copper ions revealed a strong synergistic behavior with Me[down2]NNMe[down2] as well as with similarly dimethylated compounds. In contrast, cytotoxicity of Triapine and derivatives with only mono-methylations was found to be strongly reduced by addition of copper ions. A possible explanation for the enhanced anticancer activity with copper ions is the formation of reactive oxygen species, which was found to be significantly increased with the synergistic compounds. However, the highly increased cytotoxicity of Me[down2]NNMe[down2] to the nanomolar range cannot be solely explained by its copper-chelating and ROS producing abilities, as derivatives that were even less active than Triapine also showed synergism and ROS production in combination with copper ions. In conclusion, our study gave insights into the coherences between methylation of Triapine, nanomolar cytotoxicity and synergism with copper(II) ions and revealed that the latter seems not (solely) responsible for the increased cytotoxic activity of Me[down2]NNMe[down2] into the nanomolar range.

7 Axl turns transforming growth factor-[beta] signaling to pro-oncogenic functions in hepatocellular carcinoma

Wittmann, P., Bierbaumer, L., Reichl, P., Huber, H., Mikulits, W.

Medical University of Vienna
philipp.wittmann@meduniwien.ac.at

Background and aims: Hepatocellular carcinoma [HCC], the most common form of liver cancer, is the third most lethal cancer worldwide. The epithelial-mesenchymal transition [EMT] describes the transformation of epithelial cells to a de-differentiated phenotype and plays a central role in the invasion and metastasis of HCC cells. EMT is mostly regulated by transforming growth factor [TGF]-[beta] signaling which induces various tumor-promoting pathways in advanced HCC. Furthermore, TGF-[beta] signaling is known to show both tumor-suppressing and tumor-promoting functions depending on HCC progression. Yet, little is known about the molecular mechanisms responsible for this switch in functions.
Methods: Protein and transcript regulation of Axl and TGF-[beta] signaling in human HCC cell lines were analyzed by immuno blotting and quantitative PCR, respectively. Immunoprecipitation was used to examine protein-protein interaction. Cell migration was assessed by wound healing and Transwell assays. Knockouts of Axl protein were generated by CRISPR/Cas9. Re-expression of truncated and wild-type Axl was performed by lentiviral transmission.
Results: We show that the receptor tyrosine kinase Axl is upregulated and activated in mesenchymal HCC cells. Activation of Axl through its ligand Gas6 collaborates with canonical TGF-[beta] signaling. Axl phosphorylates the Smad3 linker region at S213 via c-Jun-N-terminal kinase, ultimately leading to autocrine TGF-[beta] signaling and activation of pro-metastatic TGF-[beta] target genes. In this study, we elucidated the interplay of Axl and TGF-[beta] signaling and the influence of a complete Axl knockout or truncated receptor re-expression on the down-stream signaling of Axl.
Conclusions: Taken together, we show that TGF-[beta] regulates Axl signaling upon HCC progression which further activates autocrine and pro-metastatic TGF-[beta] functions, suggesting that the Axl/TGF-[beta] signaling axis is a promising target to combat HCC.

8 Characteristics of Lymphangiogenesis in Melanoma

Wenzina, J., Puujalka, E., Petzelbauer, P.
Department of Dermatology, Skin and Endothelium Research Division (SERD), Medical University of Vienna, Austria
judith.wenzina@meduniwien.ac.at

Melanoma is a malignant tumor of melanocytes and is responsible for 75% of skin cancer deaths. The overall survival of patients with metastatic melanoma has only in the last few years shown modest improvements due to pharmaceutical intervention. However, once the tumor cells switch from a non-invasive to an invasive phenotype, they can induce lymphangiogenesis and spread to other parts of the body. One marker which is upregulated and – amongst others – responsible for lymphangiogenesis is Vascular Endothelial Growth Factor C (VEGF-C). The expression levels of VEGF-C can predict the probability of metastasis to sentinel lymph nodes, but the intracellular regulation of this marker is poorly characterized.
By correlating the mRNA expression levels of a set of 22 melanoma cell lines, we found a significant negative correlation between VEGF-C and Microphthalmia-associated Transcription

Factor (MITF) expression. This finding was confirmed by data mining in GEO databases of human melanoma Affymetrix arrays.
We found the JNK and the p38 pathway to be responsible for the inverse regulation of VEGF-C and MITF. Activation of JNK results in a VEGF-C[uplow]/MITF[uphigh] phenotype, those cells are highly proliferative but show low mobility whereas predominant p38 signaling results in a VEGF-C[uphigh]/MITF[uplow] phenotype - corresponding to a slowly cycling and highly mobile tumor cell.
In conclusion, the relative JNK and p38 activities determine the lymphangiogenic potential of melanoma. VEGF-C and MITF serve as biomarkers for the respective JNK and p38 activities and may be used to predict the risk of lymphangiogenesis and metastasis in melanoma.

9 Three-Dimensional Direct contact Sandwich Co-culture of Endothelium on Smooth muscle cells - Modeling an Intact Blood vessel wall.

Ganesan, MK. (1), Finsterwalder, R. (1), Leb, H. (1), Resch, U. (2), De Martin, R. (2), Petzelbauer, P. (1)
(1) Skin & Endothelium Research Division, Department of Dermatology, Medical University of Vienna, (2) Department of Vascular Biology and Thrombosis Research,Med. Univ. Vienna Center of Physiology and Pharmacology.
minu.ganesan@meduniwien.ac.at

ECs (Endothelial cells) and SMCs (Smooth muscle cells) co-cultured in a three-dimensional environment make an ideal cell culture model to study vessel wall pathologies in a controlled environment. A continuous monolayer of ECs that are closely in contact with the underlying quiescent smooth muscle cells and an extracellular matrix developed within the system are necessary for the co-culture to be precisely tissue like. We have developed a three dimensional sandwich co-culture (3D SW-CC) model of Human Umbilical Vein Endothelial Cells (HUVECs) on Human Umbilical Artery Smooth Muscle Cells (HUASMCs) and have validated its likeness to an intact vessel wall in terms of morphology, extracellular matrix composition, quiescence and response to pro-thrombotic stimuli. SMCs within the sandwich were spindle shaped and showed increased contractile marker gene expression and the ECs formed confluent monolayer with continuous cell/cell contacts on the SMCs. Immunofluorescence staining showed that the ECs and SMCs formed two distinct yet close in contact cell layers. The 3D SW-CC had an autogenous extracellular matrix. The SW-CC showed significantly less cell proliferation after 7 days of co-culturing. Basal mRNA expression levels of vessel wall activation and quiescence marker genes were comparable to that of an in vivo control. The addition of pro-thrombotic stimuli to the 3D SW-CC resulted in upregulation of activation marker gene expression levels, indicating that the in vitro system is not dormant but can be stimulated. Thus, this highly reproducible 3D SW-CC model of an intact blood vessel wall can be used to study vessel wall pathologies at the cellular level, in vitro.

10 Light exposure and seasonal variation of the serotonin degrading enzyme monoamine oxidase A in the healthy human brain revealed by PET

Hienert, M. (1), Spies, M. (1), Vraka, C. (2), James, G.M. (1), Hahn, A. (1), Nics, L. (2), Philippe, C. (2), Gryglewski, G. (1), Baldinger-Melich, P. (1), Kautzky, A. (1), Komorowski, A. (1), Vanicek, T. (1), Winkler-Pjrek, E. (1), Traub-Weidinger, T. (2), Wadsak, W. (2), Mitterhauser, M. (2), Hacker, M. (2), Kasper, S. (1), Winkler, D. (1), Lanzenberger, R. (1)
1 Department of Psychiatry and Psychotherapy, Medical University of Vienna, Austria 2 Department of Biomedical Imaging and Image-guided Therapy, Division of Nuclear Medicine, Medical University of Vienna, Austria
marius.hienert@meduniwien.ac.at

Objectives: Monoamine oxidase (MAO) A is the key enzyme responsible for the oxidative degradation of several biogenic amines including serotonin in the human brain. A previous positron emission tomography (PET) study revealed elevated MAO-A levels in patients with depressive symptoms, potentially leading to lower serotonergic neurotransmission in these subjects. Seasonal changes in mood, like blues during the dark time of the year, are common within healthy controls living in areas of high latitude. We aimed to demonstrate a light dependent seasonal difference in MAO-A distribution volume (VT) in a healthy study population.
Methods: 16 healthy subjects (mean age: 37; 14 female) underwent 2 PET scans, one in summer and one in winter, using the radioligand [11C]harmine. PET images were co-registered to structural magnetic resonance imaging scans and normalized using SPM12. Quantification of MAO-A VT was carried out in PMOD 3.509 using Logan plots for 13 regions of interest. Statistical analysis was performed in SPM12 using Pearson´s correlation between regional MAO-A VT and the cumulated amount of individual exposure to global radiation (total light intensity) during the days (1-30) before the PET scans.
Results: We found significant negative correlations between cumulated global radiation and MAO-A VT in the amygdala, anterior cingulate cortex and caudate nucleus ($r=-0.561$, $r=-0.550$ and $r=-0.569$; $p<0.05$, highest correlation coefficient for the period of 5 to 14 days) in winter PET scans only.
Conclusions: These findings suggest an increase in MAO-A during winter associated with light deprivation in regions implicated in previous imaging studies on depression. Although the subjects in our study population showed no signs of depressive symptoms these results shed light on the often experienced “seasonality” in healthy people. The lack of a relation between MAO-A and light exposure during the summer months might be explained by a ceiling effect.

11 Patterns of microglia expression in experimental and human Alzheimer’s disease

Zrzavy, T (1), Wimmer, I. (1), Butovsky, O. (2), Weiner, H.L. (2), Lassmann, H. (1)
(1) Center for Brain Research, Medical University of Vienna; (2) Ann Romney Center for Neurologic Diseases, Department of Neurology, Brigham and Women’s Hospital, Harvard Medical School
tobias.zrzavy@meduniwien.ac.at

Microglia activation has been suggested to play a major role in the pathology of Alzheimer’s disease. However detailed information of patterns of microglia activation in experimental AD models in comparison to human AD is sparse.

We have performed a comparative study on microglia activation in the APP/PS1 mouse model of AD in comparison to human AD by using markers for homeostatic microglia (P2rY12, 4D4 and Tmem119), markers for phagocytic and cytotoxic microglia activation (Mac3, CD68 and p22phox).
In the mouse model microglia activation was restricted to amyloid (Aß plaques) characterized by a loss of homeostatic markers and upregulation of phagocytic markers while in the non plaque affected cortex microglia retained their homeostatic marker profile as it was seen in age matched controls.
In human AD the plaque associated microglia revealed an activation profile similar to that seen in the mouse AD model. However the AD pathology occurred on a background of general and diffuse microglia activation in the entire cortex with prominent expression of molecules associated with phagocytosis and oxygen free radical production. Their diffuse activation pattern was qualitatively similar but quantitatively less pronounced in human aged matched controls and was associated with diffuse neurodegeneration in the cortex and with peripheral immune activation at the time of death.
Our studies provide evidence for fundamental differences in microglia activation between mouse models of AD and human AD, which have to be considered in studies especially related to neurodegeneration in this disease

12 A neuropeptidergic trace of acute stress in a central fear circuit switches active to passive coping strategies

Pliota, P. (1), Grössl, F. (1), Griessner, J. (1, 2), Böhm, V. (1), Valenti, O. (2), Pasieka, M. (3), Lendl, T. (4), and Haubensak, W. (1)
(1) Research Institute of Molecular Pathology, Vienna, Austria, (2) Medical University of Vienna, Austria, (3) Vienna Biocenter Core Facilities GmbH, Vienna, Austria, (4) Gregor Mendel Institute of Molecular Plant Biology GmbH, Vienna, Austria
(1) Research Institute of Molecular Pathology, Vienna, Austria
pliota@imp.ac.at

Survival of an animal strongly relies upon optimizing its behavioral strategies based on previous experiences. Threat-related behavioral plasticity is perhaps the etiologically most basic and biomedically most relevant example. Animals learn from aversive experiences that specific cues predict danger and best be avoided. However, in the absence of such discrete cues the threat remains unpredictable. In such cases the animal adapts by switching from active to more passive behaviors. Here, we investigated the neuronal basis of this phenomenon and explore general principles of behavioral plasticity in the brain.

To this end, we developed a behavioral paradigm to study such acute stress driven changes in behavioral strategies in mice in subsequent environmental challenges. We used molecular, pharmaco-, optogenetic and electrophysiological methods as well as Ca2+ imaging to dissect the underlying mechanisms in central amygdala (CE) - a key structure for fear behaviors. We have identified the paraventricular thalamus (PVT), a nucleus known to be involved in stress response, as one of the major inputs to the CE. Using site-specific lesions and projection-specific optogenetic manipulation we identified a role for the PVT-CE connection in behavioral effects of acute stress. Furthermore, by combining electrophysiology with optogenetics, we were able to

demonstrate that PVT asymmetrically innervates two antagonistic circuits in CE. Together with data from Ca2+ imaging in awake behaving animals, our results suggest that acute stress experience activates PVT-CE projections and releases a local neuropeptidergic signal. This in turn modulates local circuit dynamics and switches behavioral strategies from active to passive.

13 Depression in acute intermittent porphyria – pathogenic principles and neurobiological mechanisms

Berger, S., Pollak, D.D.
Medical University Vienna
stefanie.berger@hotmail.com

Acute intermittent porphyria (AIP), an autosomal dominant inborn error of heme biosynthesis, is due to the half-normal activity of hydroxymethylbilane synthase (HMBS). AIP is characterized by life-threatening acute neurovisceral attacks that are triggered by various factors (drugs, hormonal changes, fasting) that induce the hepatic expression of aminolevulinic acid (ALA) synthase 1 resulting in accumulation of the neurotoxic porphyrin precursors, ALA and porphobilinogen (PBG). While neuropsychiatric conditions like depression are reported in up to 50% of AIP patients, the underlying pathogenic mechanisms remain unclear.
We aim to investigate the emotional disturbances and underlying neurobiological mechanisms using an AIP knock-in (KI) mouse model. These mice are homozygous for the human HMBS mutation and have elevated ALA and PBG levels. Initial efforts revealed enhanced depression-like behavior in the KI mice in standard behavioral tests and alterations in general behavioral functions. While no gross pathohistological disturbances were revealed in KI mouse brains, the rate of proliferation of newborn cells in the hippocampal dentate gyrus of KI mice was severely hampered, a cellular characteristic strongly linked to depression.
The next step will be directed against the investigation of differentiation and survival of adult hippocampal cells.
We will also monitor electrophysiological function in the hippocampus of the KI and WT mice.
In the final step we seek to unravel the molecular mechanism involved in the behavioral and neurogenic deficits in AIP focusing on evaluating Gamma-aminobutyric acid (GABA) receptor density, subtype expression and signaling; ALA has been shown to specifically interact with elements of the GABAergic neurotransmitter system, which is highly implicated in the pathophysiology of mood disorders.
The proposed research has the potential to further our understanding of the neural mechanisms underlying and mediating depression in AIP

14 Retinal pathology in experimental Neuromyelitis optica: Axonal damage and loss of aquaporin-4

Zeka, B. (1), Hastermann, M. (1), Kaufmann, N. (1), Schanda, K. (2), Pende, M. (3), Misu, T. (4), Rommer, P. (5), Fujihara K. (4), Dahle C. (6), Leutmezer F. (5), Reindl M. (2), Lassmann, H. (1), Bradl M (1).
(1) Medical University Vienna, Department of Neuroimmunology, Center for Brain Research, Austria, (2) Innsbruck Medical University, Clinical Department of Neurology, Austria, (3)

Medical University Vienna, Section for Bioelectronics, Center for Brain Research, Austria, (4) Department of Neurology, Tohoku University Graduate School of Medicine, Sendai, (5) Medical University Vienna, University Hospital for Neurology, (6) Department of Clinical and Experimental Medicine, Faculty of Health Sciences, Linköping University, Linköping, Sweden
bleranda.zeka@meduniwien.ac.at

Neuromyelitis optica (NMO) is an inflammatory, astrocytopathic disease of the CNS. Pathogenic antibodies (NMO IgG) against the water channel aquaporin-4 (AQP4) target astrocytes and cause complement-dependent and antibody-dependent damage, predominantly in the optic nerves and spinal cord. However, also astrocytes and Müller cells in the eye express AQP4. This raised the question whether also these cells could be targeted in NMO, especially since OCT studies already revealed microcystic inner nuclear layer abnormalities and retinal nerve fiber layer (RNFL) thinning in NMO patients. We addressed this question in experimental NMO (ENMO), induced by peripheral injection of NMO IgG during AQP4-specific T cell-mediated experimental autoimmune encephalomyelitis (EAE) in Lewis rats. We found AQP4-specific T cells in the eyes, mainly in the optic nerve head and in the retina. At these sites, primary axonal dysfunction/damage was observed, as indicated by beta amyloid precursor protein (b-APP) positive spheroids/end bulbs. This was seen independently of the presence of axonal dysfunction/damage in the optic nerve, and also independently of the presence or absence of NMO IgG, suggesting that they were induced by the action of T cells. In the presence of NMO IgG, we observed loss of AQP4 in the outer plexiform layer (OPL), but not in the RNFL. Since Müller cell processes are predominantly found in the OPL and astrocyte processes mainly in the RNFL, we conclude that retinal Müller cells are targeted by pathogenic AQP4-specific antibody in ENMO but retinal astrocytes are spared.

15 The SOST-knockout mouse model is more similar with Sclerosteosis in humans than previously known

Schwarze, U.Y. (1,2), Dobsak, T. (2,3), Bookstein, F.L. (4,5), Gruber, R. (1,2,6)
(1) Department of Oral Biology, Medical University Vienna, Austria (2) Austrian Cluster for Tissue Regeneration (3) Department of Oral Surgery, Medical University Vienna, Austria (4) Department of Anthropology, University of Vienna, Austria (5) Department of Statistics, University of Washington, Seattle, USA (6) Department of Preventive, Restorative and Pediatric Dentistry, University of Bern, Switzerland
uweyacine.schwarze@meduniwien.ac.at

The inactivation of the SOST gene and therefore absence of sclerostin leads to pathologies (Sclerosteosis, Van Buchem disease), with high bone mass and density. Sclerosteosis is more severe, with gigantism, facial distortion, mandibular prognathism, cranial nerve palsy and in extreme cases, medulla oblongata compression as symptoms. Sclerostin negatively regulates the Wnt signalling pathway which regulates new bone formation. Artificial inhibiting of sclerostin could lead to new therapies against bone loss. Until now only higher bone mass and density was known in SOST-knockout (KO) mouse skulls, this study works to learn more.
Geometric morphometrics was used to analyse Symptoms of Sclerosteosis in a group of six SOST-KO mice and six respective WT control. [my]CTs of the head were obtained and surfaces

with 27 landmarks each reconstructed. Centroid size, PCAs in shape and form space, asymmetry were computed and, dental and skeletal mandibular prognathism were graphically assessed.
Comparing SOST-KO and WT mice, SOST-KO mice show greater centroid size, more asymmetry, dental and skeletal mandibular prognathism and a smaller diameter of the foramen magnum. The PCAs distinguish between groups with more highly curved calvaria in the SOST-KO group.
The results are similar to the symptoms found in Sclerosteosis patients. SOST-KO mice are larger in size, have deformations, a tendency for dental and mandibular prognathism and relatively smaller nerve openings. SOST-KO mice thus have more in common with sclerosteosis patients than previously known, showing more effect comparability between mouse and human studies.

16 Effect of prolyl hydroxylase inhibitors-loaded collagen membranes on osteoclastogenesis and osteoblastogenesis

Edelmayer, M. (1,2,3), l-Habbal, D. (2,3), Pensch, M. (1,3), Janjić, K.(2,3), Agis, H. (2,3,)
(1) Department of Oral Surgery, Medical University of Vienna, Austria (2) Department of Conservative Dentistry and Periodontology, Medical University of Vienna, Austria (3) Austrian Cluster for Tissue Regeneration
michael.edelmayer@meduniwien.ac.at

In oral surgery collagen membranes are used for guided bone regeneration (GBR) to support regeneration of hard tissue. This strategy is based on the endogenous healing capability of the respective tissue. Prolyl hydroxylase (PHD)-inhibitors induce a proangiogenic response and therefore could be applied to optimize periodontal and surgical treatment. In this study, the effect of PHD inhibitors dimethyloxalylglycine (DMOG) and deferoxamine (DFO), released from loaded collagen membranes on osteoclastogenesis and osteoblastogenesis, was evaluated. DMOG or DFO were applied onto collagen membranes and lyophilized. Release studies were performed and the supernatants were taken after 1, 3, 6, 24, and 48 hours. Using these generated supernatants, the effect on osteoblast- and osteoclast-precursor cells was evaluated. The impact on osteoclasts was evaluated with RAW 264.7 cells based on the number of multinuclear tartrate-resistant acid phosphatase positive cells. The impact on osteoblasts was evaluated with MC3T3-E1 cells based on alkaline phosphatase staining and measurement. In addition, cell proliferation and metabolic activity was assessed based on BrdU and MTT assays with both cell lines. VEGF production was evaluated using ELISA. Supernatants taken in the first hour from collagen membranes loaded with DMOG or DFO reduced osteoclastogenesis. Osteoblastogenesis was not reduced significantly. Cell proliferation and metabolic activity of RAW264.7 and MC3T3-E1 cells were inhibited by DFO but not by DMOG. In RAW264.7 culture, VEGF production was increased only by DMOG but not by DFO. In MC3T3-E1 culture, DMOG and DFO both increased VEGF production. Our findings show divergent effects of DMOG- and DFO-loaded collagen membranes during osteoclastogenesis and osteoblastogenesis. Future studies are needed to evaluate if the increase in VEGF together with the inhibitory effect on osteoclasts can have a positive influence on oral tissue regeneration in periodontal and oral surgery.

17 Clinical comparison of treatment modalities in case of 1st implantation versus reimplantation: a 3-year follow-up investigation

Ubaidha Maheen, C. (1), Seemann, R. (2), Rausch-Fan, X. (1), Watzek, G. (3), Mailath-Pokorny, G. (3), Haas, R. (3), Busenlechner, D. (3), Fürhauser, R. (3), Pommer, B. (3).
(1) Bernhard Gottlieb School of Dentistry, Medical University of Vienna, Austria (2) Department of Maxillofacial Surgery, Medical University of Vienna, Austria (3) Academy for Oral Implantology, Vienna, Austria
ceeneena@yahoo.com

Background: Reimplantation in previously failed sites is a challenging therapeutic issue to the clinician; and only limited information is available about the survival rate of reimplants.
Aim: The aim of this retrospective study was to compare treatment modalities and survival rates with 1st implantation and reimplantation.
Materials and Methods: This study was conducted on 3599 patients with 10,779 implants from 2004 to 2012. 10617 implants were evaluated in the 1st implantation group and 162 implants in the reimplantation group. The 3-year survival rates were computed using the Kaplan-meier method, then compared via log rank test. Different treatment modalities were evaluated by Fisher tests.
Results: Overall 1st implantation group survival was 97% and reimplantation group survival was 88%. There were highly significant difference between survival rate of 1st implantation and reimplantation ($p<0.001$), timing of various bone grafting ($p=0.020$), healing modality ($p<0.001$) and timing of different provisionalization ($p=0.001$) in relation to implant placement in between 1st implantation and reimplantation. There were no significant difference between timing of soft tissue grafting ($p=0.170$) and timing of different loading protocol ($p=1.000$).
Conclusion: Submucosal healing modality is the most preferred treatment in reimplantation, and reimplantation increased the risk of implant failure by 9- fold.

18 Identification Of Factors Leading To Total Knee Replacement - Data from the Osteoarthritis Initiative (OAI)

Reumann S (1), Boudreau R (2), Hitzl W (3), Holinka J (1), Hobusch G (1) Windhager R (1), Cotofana S (4,5)
1 Department of Orthopedics, Medical University of Vienna, Vienna, Austria 2 Center for Aging and Population Health, Dept. of Epidemiology, University of Pittsburgh, PA 3 Research Office, Biostatistics, Paracelsus Medical University, Salzburg, Austria 4 Department of Anatomy, Ross University School of Medicine, Roseau, Commonwealth of Dominica 5 Institute of Anatomy, Paracelsus Medical University Salzburg & Nuernberg,Salzburg, Austria
s.reumann@yahoo.com

Objective To investigate the driving clinical and imaging based factors leading to TKR surgery in patients with knee osteoarthritis.
Methods 165 participants were identified from the Osteoarthritis Initiative (OAI) who received Total Knee Replacement (TKR) during a 4-year period. Annual publicly available patient data were obtained in the visit before TKR, 1, 2, 3 and 4 years before TKR. Between these time points we compared the participant's quality of life, WOMAC total score, WOMAC pain sub

score, knee pain intensity score and Kellgren and Lawrence Grades classification. To estimate the relation between clinical parameters‘ change and structural progression we defined a “clinical/structural change index" (CSCI).
Results Median KL grades increased each successive year prior to TKR ($p<0.0046$). For measures of quality of life, WOMAC pain score, WOMAC total score a significant change was observed in the year prior to TKR ($p<0.0001$). Scores for pain intensity changed significantly starting 2 years prior to TKR ($p=0.014$).
Scores for the CSCI for QoL, WOMAC pain sub score, WOMAC total score and pain intensity were 1.05, 4.4, 3.57 and 1.9 respectively. Additionally, we created a prediction model with a sensitivity and specificity of 70%.
Conclusion The results of this study indicate that the driving factors for surgery seem to be based majorly on patients quality of life and knee specific pain and functional scores. Albeit advanced KL grades are a strong indicator for surgery, clinical factors were more predictive. A CSCI with a value of more than 1 might potentially be a new and valuable indicator when trying to estimate a patient’s need for TKR.The prediction model might be useful in a primary care setting to assess a patient's future need for TKR.

19 Comparison of different metal artifact reduction techniques in MDCT and CBCT for titanium and zirconia dental implants by post-processing in-vitro

Beck, F., Zechner, W., Hirtler, L., Schedle, A., Dobsak, T., Ulm, C., Gahleitner, A., Homolka, P.
University Clinic of Dentistry Vienna, Medical University Vienna
florian.beck@meduniwien.ac.at

Introduction: After implant placement intraoral x-ray is the most commonly used radiographic technique for follow-up observations and for the diagnosis of peri-implantitis. This radiographic technique faces one major limitation; marginal bone support of the implant cannot be evaluated from an oral and facial aspect due to its two-dimensional nature. Therefore computed tomography has gained popularity for three-dimensional evaluation, however studies have reported contradicting results in regard to accuracy in the determination of bone loss close to dental implants. Beam-hardening and quantum starvation account for the strongest artifacts in computed tomography and thus make the evaluation of hard tissue around implants imprecise. The aim of this study is to compare the accuracy of two MDCTs and two CBCT scanners with different types of implants (Ti, TiZr, ZrO) by applying different methods for artifact reduction.
Material and Methods: A human edentulous mandible that was provided by the Institute of Anatomy (Medical University of Vienna) served as a model for implant placement. The mandible was scanned in a MDCT system (Somatom Sensation 10, Siemens AG, Forchheim, Germany), manually segmented (AMIRA(R) software, VSG, Burlington, MA, USA) and saved in STL format (stereolithography) to allow fusion with a head phantom to be produced on a 3D printer. The head phantom model was created in ARMIRA(R) allowing insertion of the mandible with various implants. In total, four implants (three titanium-zirconium alloy (n=3) and one zirconia implant) were inserted according to the manufactures protocol (Straumann AG, Basel, Switzerland). Then the mandible was inserted in the head phantom substituting soft tissue by gelatine to achieve proper soft tissue imitation.
Results: As this study is still in process, preliminary results cannot be shown at present time.

20 Long-term investigation of retinal changes in a VLDLR mouse model using multi-functional OCT

Augustin, M. (1), Fialova, S. (1), Himmel, T. (2), Glösmann, M. (2), Harper, D.J. (1), Lengheimer, T. (3), Plasenzotti, R. (3), Pircher, M. (1), Hitzenberger, C.K. (1), Baumann, B. (1)
(1) Center for Medical Physics and Biomedical Engineering, Medical University of Vienna, Vienna, Austria; (2) Core Facility for Research and Technology, University of Veterinary Medicine Vienna, Vienna, Austria; (3) Division of Biomedical Research, Medical University of Vienna, Vienna, Austria
marco.augustin@meduniwien.ac.at

Animal models play an important role in preclinical research of ophthalmic diseases. Multi-functional optical coherence tomography [mufuOCT] is a non-invasive imaging method to study retinal changes with threefold contrast – reflectivity data, polarization sensitive [PS] imaging and OCT angiography [OCTA].
A PS-OCT system was utilized to image the retina of both eyes in 8 very-low-density-lipoprotein receptor [VLDLR] mice every 4 to 6 weeks within the age of 28 to 326 days. 3D datasets comprising 512 A-scans, 400 B-scans with 5 repetitions at each B-scan position, covering a field of view of 28°×28°, were acquired. OCTA, based on motion contrast within a set B-scans, was used to visualize the vasculature in the mouse retina. Averaging each set was used for speckle noise reduction in the PS contrast and the reflectivity images. Degree-of-polarization-uniformity [DOPU] images were calculated in a 3D kernel of 3×9×5 (z×x×t). A retinal layer segmentation algorithm was implemented to segment 5 contours in the retina. Contrast specific projections within different slabs of the retina, e.g. minimum DOPU projection, were used to highlight various aspects during disease progression over time and enabled a long-term evaluation of the image data.
Retinal lesions were present in all eyes at the initial measurement in the reflectivity images. A trend of decreasing retinal thickness was observed during the year of observation. Vascular changes such as anastomosis of retinal and choroidal vessels were identified using OCTA. Depolarizing deposits due to detached pigmented tissue were identified in the outer nuclear layer region close to lesion sites. Histological sectioning was performed with 3 mice at the endpoint to confirm the in-vivo findings.
This works demonstrates the potential of mufuOCT for long-term investigations of mouse models of ophthalmic diseases. The VLDLR mouse model showed severe pathological retinal changes in each of the investigated contrast channels.

21 Multimodal assessment of TMS-induced acute effects

Tik M., Woletz M., Navarro de Lara L., Sladky R., Hoffmann A., Hummer A., Windischberger C.
Medical University of Vienna, Center for Medical Physics and Biomedical Engineering, Wien
martin.tik@meduniwien.ac.at

Transcranial magnetic stimulation (TMS) has become a promising therapeutic treatment and neuroscientific research tool, yet how it works is not fully understood. We developed a dedicated

MR coil array positioned beneath the TMS coil that allows for concurrent stimulation and imaging and flexible positioning while ensuring TMS efficiency, high MR sensitivity and the use of parallel/multiband imaging sequences.
Here we aimed to explain acute effects of TMS over left DLPFC using a new online TMS/fMRI setup in combination with advanced imaging and neuronavigation methods. Resting State functional connectivity and DTI Tractography shall further explain resulting activation patterns at a network level.
The study was performed on a 3T Tim Trio scanner (Siemens, Erlangen, Germany). The TMS system used included a MagProX100 stimulator and MRi-B91 MR-compatible TMS coil (Magventure, Farum, Denmark). Five right-handed female subjects (age: 28.6 ± 4.3 years) were examined. Functional images were acquired using EPI (echo-planar imaging) sequence with TR/TE=1000/33ms, 28 slices, 1.5x1.5x3mm³. FMRI data analyses were performed using SPM12. The design matrix comprised four regressors representing different stimulation amplitudes. DTI tractography was performed using DSI Studio.
TMS led to intensity-dependent activation increase in the left DLPFC and the contralateral DLPFC (Fig. 2): Higher stimulation intensities evoked higher bilateral activation. ACC showed a more complex response pattern.
Resting-state functional connectivity maps using the stimulation target as seed-voxel showed a network very similar to the TMS-derived activation pattern (Fig. 3). DTI tractography provided additional information about the axonal connection between the left DLPFC stimulation site and the right DLPFC activation cluster.
Here we show dose-dependent, local (at stimulation site) activation changes and remote alterations in associated networks, as confirmed by RS & DTI connectivity.

22 Non-linear B-spline image registration for three-dimensional reconstruction of brain tissue

Anner, P., Passecker, J., Klausberger, T., Dorffner, G.
Medical University of Vienna
philip.anner@meduniwien.ac.at

Generating three dimensional (3D) models of paraformaldehyde-fixed sections of brain tissue is a major task in current research. The models can be used for spatial identification of entities such as cell somata or blood vessels. Histological entities are cut in thin slices and cells can be further identified by immunohistological labeling. The slices are usually 3D scanned with a confocal microscope resulting in a z-stack. Image registration is the process of aligning, transforming, and merging the z-stacks to a 3D model. We evaluated linear and non-linear image registration techniques within the rats' prefrontal cortex. The cutting process introduced inhomogenities on the upper and lower borders of the brain slices (70 um thickness), which resulted in local loss of information. This was accounted for by applying maximum intensity projection (MIP). As a measure of similarity between the images of the consecutive slices we used the squared intensity differences of the pixels. The measure was enhanced by manually selected reference points. Linear transformations and affine transformations were evaluated, but resulted in non-satisfactory results. Non-linear distortions caused by fixation, cutting and the mounting on the glass probe of the tissue have to be accounted in the image registration technique. A simulation study was performed to assess the image registration techniques based on images of one physical

slice. Images with a gap of 21nm were selected and the image to be registered was spatially deformed. The result of the image registration process was then compared to the original (non-deformed) image. Three dimensional histological reconstructions using linear and B-spline transformations were generated. The image registration was assessed visually by focusing on entities (cell somata, blood vessels and dendrites) that appeared on consecutive sections. Using B-spline transformations we could remove the distortions in order to achieve a consistent 3D model..

23 Monitoring of physical activity in patients with a left ventricular assist device implanted

Gross, C., Haller, M., Zimpfer, D., Schima, H., Moscato, F.
Medical University Vienna
christoph.gross@meduniwien.ac.at

Monitoring of daily physical activity is an emerging tool to assess health status both in normal and pathologic conditions. Such monitoring can be also important in patients with a left ventricular assist device (LVAD) implanted. This study aims at the validation of activity detection based on accelerometer data and at its first application to monitor post-implant patient activity.
A 3-axial accelerometer was embedded in a previously developed recording device for LVAD data, which is placed in the patient's shoulder-bag. The measured signals (sampled at 10Hz) provided a binary classification of activity/rest based on device position and acceleration magnitude. During ambulatory visits the accelerometer activity was compared to data recorded in a log-sheet including the time course of active and resting periods. Once validated the post-operative course of total daily activity (min/day) was also analyzed.
Preliminary validation of the accelerometer activity and protocolled activities was performed with 17 datasets from 11 patients and resulted in a sensitivity of 96.3[+-]3.1% and a specificity of 93.6[+-]6.8%. An average of 152 days of activity data were recorded in 7 patients within the first 200 days post-implant. At post-operative days 50, 100, 150, 200 average daily physical activity was 60, 77, 88, 65 min/day. Two rehospitalizations in one patient were correlated with a drop in detected activity.
Activity derived from the accelerometer can be useful to examine LVAD therapy. Combined with hemodynamic pump monitoring, it will give a more comprehensive picture of the interaction between LVAD and the remaining cardiac function during daily living.

24 The effect of the endurance training on plasma lipoproteins and cardiovascular capacity in older marathon runners

Batmyagmar, D. (1), Haslacher, H. (2), Ponocny-Seliger, E. (3), Kundi, M. (1), Lehrner, J. (4), Winker, R. (3)
(1) Department of Public Health, Medical University of Vienna, Austria (2) Department of Laboratory Medicine, Medical University of Vienna, Austria (3)Health and Prevention Center, Sanatorium Hera, Vienna, Austria (4) Department of Neurology, Medical University of Vienna, Austria

n1142422@students.meduniwien.ac.at

The positive effect of physical exercise on high- density lipoprotein (HDL) and on reductions of low-density lipoprotein (LDL), total cholesterol and triglycerides (TC) has been associated with a reduction in risk of atherosclerosis and cardiovascular diseases. This effect depends on the duration, frequency, intensity and type of the activity and on the overall levels of lipoproteins that in turn depend on individual characteristics, such as gender, age, BMI and dietary habits. Previous research explored the relationship between exercise intensity and plasma lipids and lipoprotein levels. Nevertheless these findings are controversial. Thus, we aim to investigate whether intensive endurance training impacts on plasma lipid levels and cardiovascular capacity in a prospective cohort of elderly marathon runners.
We studied participants from the Austrian Prospective Cohort Study in Cognitive Function of Elderly Marathon-runners (APSOEM, athletes n= 50 [♀=4], controls n=49 [♀=5]). Data (physical examination, treadmill test, blood test) were collected at baseline and after a 3 years follow-up period.
The mean BMI was 27.8±5.6 kg⁄m² (m2=m[up2]) in controls and 23.6±3.0 kg⁄m² (m2=m[up2]) in athletes, respectively ($p<0.001$). In the treadmill assessment, the elderly marathon group performed significantly better than controls ($p<0.001$). Athletes had lower TC levels ($p=0.005$) and higher HDL ($p=0.013$) compared to controls. No differences were found for cardiovascular function except overall capacity. However, a simple score containing three baseline routine biochemical results (HbA1c (=HbA[down1c]), red blood count, ASAT) allowed for acceptable estimation of future cardiovascular arterial indices in the athletes, but not in controls.
Athletes had better health profiles compared to controls in terms of BMI, HDL, less TC and higher physical capacity. Moreover, our results indicate that among athletes future cardiovascular health might be predictable by biochemical parameters.

25 Epigenome-Wide RNAi Screen Identifies an Alpha to Beta Cell Transdifferentiation Factor

Casteels, T., Li, J., Kubicek, S.
CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria
tcasteels@cemm.oeaw.ac.at

Pancreatic β cells represent the sole source of insulin in the human body. They closely interact with their neighbouring cells in the islets of Langerhans, namely the glucagon-secreting α cells, to maintain normoglycaemia. Hence, loss of these β cells, as observed in Type I Diabetes (T1D), results in deregulated glucose homeostasis and severe hyperglycaemia. This underscores the need for a novel insulin cell source in T1D patients. The close developmental link between α- and β-cells, evident via their common Ngn3+ progenitor, make α-cells a promising candidate. Hence, we are attempting to induce β cell characteristics in α cells, in the hopes of replenishing β cell mass and gaining a broader understanding of chromatin-mediated transdifferentiation. We conducted a chromatin-focused short hairpin RNA screen on the murine α cell line, aTC1, in search of proteins repressing β cell markers. The viral library targeted over 300 potentially

druggable chromatin factors. The cells were then screened for changes in their transcription profile, with a particular focus on increased insulin (Ins2) expression. The strongest hit was a protein involved in post-transcriptional modifications. RNAseq results show a general upregulation in β cell markers, including Iapp, Gck, Pax4 and Ins2, upon knockdown in aTC1 cells. Knockout experiments reveal the essentiality of the gene for α cell survival and proliferation. Its function appears to be conserved in human islets, in which its knockdown induces a significant upregulation of Pax4 transcription. Currently we are attempting to functionally characterize the encoded protein via affinity proteomics, ChIPseq and mutagenesis experiments. Overall, we have identified a promising candidate whose knockdown in α cells yields an increase in β cell specific markers. These experiments could yield valuable information regarding transcriptional regulation in the endocrine pancreas, and potentially a new insulin cell source.

26 Organ-on-a-chip: the synovial adaptive response in arthritis.

Olmos Calvo, I. (1,2), Byrne, R.A. (1), Karonitsch, T. (1), Niederreiter, B. (1), Kartnig, F. (1), Alasti, F. (1), Holinka, J. (3), Ertl, P. (2), Kiener, H.P. (1)
(1) Department of Medicine III, Division of Rheumatology, Medical University of Vienna, Austria; (2) Faculty of Technical Chemistry, Vienna University of Technology (TUW), Vienna, Austria (3) Department of Orthopaedic Surgery, Medical University of Vienna
is.olmos.ca@gmail.com

Rheumatoid arthritis (RA) is a systemic autoimmune disease that primarily affects the synovium of diarthrodial joints. The synovium is formed by fibroblast-like synoviocytes (FLS) and serves distinct functions critical to joint homeostasis. In RA, the synovium remodels to an aggressive cell mass that destroys the articular cartilage. We hypothesize that synovial remodelling in arthritis is intricately linked to altered tissue functions in support of the perpetuation of the inflammatory destructive process in RA.
To explore mechanisms of the synovial inflammatory response in RA, we established a 3D synovial culture system on-chip. Primary human FLS were labelled with cell tracker dyes, embedded in extracellular matrix and cultured as a sphere on-chip. Two photon laser scanning microscopy was used to continuously monitor tissue formation for up to seven days.
Within few days of culture, the FLS established a lining structure at the surface of the sphere by the recruitment of sublining fibroblasts into the lining layer. Within the lining structure, the spindle shaped cells were oriented in parallel to the surface, resembling the in-vivo situation of the synovial lining. Strikingly, when stimulated with the pro-inflammatory cytokine TNF, the FLS re-organized and formed densely packed cellular aggregates in the sublining area. Thus, the 3D synovial culture system on-chip recapitulates characteristics of both, the normal as well as the diseased synovium. Intriguingly, immunohistochemistry and staining for the proliferation marker Ki-67, demonstrates increased rates of proliferation in cells at the lining layer when compared to sublining cells.
Next steps are expression profiling and gene silencing of candidate genes in order to identify molecular pathways of the deleterious inflammatory response of the synovium.

27 The environment alters the immune response to ragweed pollen

Liu, S.-H. (1), Debiasi, M. (1), Anea, C.B. (1), Karrer, G. (2), Chaturvedi, P. (3), Bellaire, A. (4), Weckwerth, W. (3), Epstein, M.M.(1)
(1) Medical University of Vienna, Department of Dermatology, DIAID, Vienna, Austria, (2) University of Natural Resources and Applied Life Sciences, Vienna, Austria, (3) University of Vienna, Department of Molecular Systems Biology, Vienna, Austria, (4) University of Vienna, Department of Botany and Biodiversity Research, Vienna, Austria

shu-hua.liu@meduniwien.ac.at

Ambrosia artemisiifolia (ragweed) is a highly invasive plant with pollen that causes allergy. Using an experimental mouse model of ragweed pollen-induced allergic disease, we sought to determine whether environmental factors e.g., climate and pollution exacerbate pollen allergy. Ragweed pollen from several sources including some collected from urban and rural areas in Austria were repeatedly administered intranasally (i.n.) to female BALB/c mice. Pollen were either untreated or treated with low pH, high temperature and various pollutants including ozone to mimic acid rain, extreme heat and drought periods, and ground and air pollution. When treated or untreated ragweed pollen suspensions (10 Âµg) were administered i.n. 6 times over a 3-week period, mice developed lung and airway inflammation, mucus hypersecretion, and high serum ragweed-specific IgG1 titres in a dose-dependent fashion. Interestingly, disease was more severe with pollen from urban compared with rural areas. Similarly, in vitro-treated pollen generated differential in vivo responses. Taken together, these data demonstrate that the environment alters the allergenicity of ragweed pollen. These results have serious ramifications on environmental health and well-being and underscore the importance of addressing climate change and air quality issues by policy makers. Further studies are necessary to elucidate the mechanism underlying the environmental effect on pollen.

28 The vitamin D hormone is a posttranslational regulator of FGF23 secretion

Bayer, J., Murali, S. K., Andrukhova, O., Erben, R. G.
University of Veterinary Medicine Vienna
Jessica.Bayer@vetmeduni.ac.at

Fibroblast growth factor 23 (Fgf23), a bone-produced hormone, plays a critical role in vitamin D and phosphate homeostasis. Human diseases with high intact circulating FGF23 (iFGF23) result in hypophosphatemia and low vitamin D hormone (1,25(OH)[down2]D[down3]). Release of iFGF23 is controlled by intracellular proteolytic processing involving the endoprotease Furin. O-glycosylation of FGF23 by the protein N-acetylgalactosaminyltransferase 3 (GALNT3) prevents cleavage of iFGF23, an action which is antagonized by the kinase family with sequence similarity 20, member C (Fam20c). The 1,25(OH)[down2]D[down3]-mediated increase in FGF23 transcription is well known. Here, we examined the role of vitamin D in posttranslational processing of Fgf23, using mice with a nonfunctioning vitamin D receptor (VDR[upÎ”/Î”]) as loss-of-function model, and wildtype (WT) C57BL/6 mice treated with 1,25(OH)[down2]D[down3] (1 Âµg/kg/day for 2 days) as gain-of-function model. All mice were kept on the rescue diet rich in calcium, phosphorus, and lactose to prevent severe

hyperparathyroidism in VDR[upÎ”/Î”] mice. The ratio of iFgf23 to C-terminal Fgf23 in the serum of VDR[upÎ”/Î”] mice was lower, whereas that of 1,25(OH)[down2]D[down3]-treated WT-mice was higher than WT or vehicle controls, respectively suggesting an involvement of vitamin D in Fgf23 protein processing. To confirm these data in vitro, we treated primary murine differentiated osteoblasts and osteocyte-like cells with 1,25(OH)[down2]D[down3] (10, 100 nM) or vehicle for 24 h. Vitamin D-treated osteoblasts showed a reduction in Fam20c and Furin and an increase in Galnt3 and Fgf23 mRNA expression compared to vehicle controls. In osteocyte-like cells, vitamin D treatment increased Galnt3 and Fgf23 but not Fam20c and Furin mRNA expression, and increased the ratio of iFgf23 to C-terminal Fgf23 in the medium. In conclusion, our data suggest that vitamin D is not only a transcriptional but also a posttranslational regulator of Fgf23 secretion.

29 Arginase I and Osteoclastogenesis

Brunner JS (1), Hofmann M (2), Saferding V (2), Paar H (1), Chen L (3), Cheng P (3), Schabbauer G (1), Blüml S (2)

(1) Medical University Vienna, Institute for Physiology, Vienna, Austria; (2) Medical University Vienna, Dpt. of Rheumatology, Vienna, Austria; (3) Bio Cancer Treatment International Ltd., Hong Kong, China

julia.s.brunner@meduniwien.ac.at

Osteoclasts are giant, multi-nucleated cells that derive from the monocyte-macrophage linage and are critically involved in bone turnover. They are further known as the main effector cells for development of age-related osteoporosis. While the role of Arginase I within certain myeloid lineages such as macrophages is well appreciated, its role within osteoclasts is relatively unknown. Our aim was therefore to investigate the importance of the enzyme in the context of osteoclastogenesis.We analyzed osteoclastogenesis of C57BL/6J or BALB/c wildtype cells in vitro in the presence and absence of recombinant Arginase I (recARG1) and its inhibitor nor-NOHA. This was complemented via qPCR analysis of relevant marker genes. We further investigated the potential of the enzyme to induce cell death via flow cytometry analysis of 7-AAD and Annexin V. In osteoclast differentiation assays, we show that Arginase I is strongly downregulated during osteoclastogenesis, suggesting involvement of this enzyme in OC differentiation. We demonstrate that addition of recArgI completely abolishes osteoclast formation without inducing cell death. The inhibitory effect of recArgI on osteoclastogenesis was completely dependent on the enzymatic function, as no decrease in osteoclast formation could be observed during combined addition of recArgI and its specific inhibitor nor-NOHA. We observed that recArgI specifically inhibits RANKL-mediated terminal differentiation of OCs, but has no effect on MCSF dependent generation of OC precursors. In line, we could show that addition of recombinant Arginase I negatively regulated the expression of classic RANKL induced osteoclastic marker genes such as TRAP and Cathepsin K. We therefore propose that

recArgI might be a potent inhibitor of osteoclastogenesis and could prove itself to be useful for the treatment of osteoclast driven diseases, such as osteoporosis.

30 Active mTORC1 signaling induces macrophage granuloma formation and sarcoidosis progression

Linke M. (1), Pham, H. T. T. (1) Katholnig, K. (1) Schnöller, T. (1) Miller, A. (2) Demel, F. (1) Preitschopf, A. (1) Kovacic, B. (1) Niederreiter, B. (3) Blüml, S. (3) Kuess, P. (4) Sexl, V. (5) Müller,M. (6) Mikula, M. (1) Weckwerth, W. (7) Haschemi, A. (2) Susani, M. (8) Hengstschläger, M. (1) Gambello, M. J. (9) Weichhart, T. (1)

(1) Institute of Medical Genetics, Medical University of Vienna, 1090 Vienna, Austria (2) Department of Laboratory Medicine, Medical University of Vienna, 1090 Vienna, Austria (3) Division of Rheumatology, Internal Medicine III, Medical University of Vienna, 1090 Vienna, Austria (4) Department of Radiation Oncology, Medical University of Vienna, 1090 Vienna, Austria (5) Institute of Pharmacology and Toxicology, University of Veterinary Medicine, 1210 Vienna, Austria (6) Biomodels Austria and Institute of Animal Breeding and Genetics, University of Veterinary Medicine, 1210 Vienna, Austria (7) Department of Ecogenomics and Systems Biology, Vienna Metabolomics Center (VIME), University of Vienna, 1090 Vienna, Austria (8) Clinical Institute of Pathology, Medical University of Vienna, 1090 Vienna, Austria (9) Department of Human Genetics, Emory University, Atlanta, Georgia 30322, United States.

monika.linke@meduniwien.ac.at

Aggregation of hypertrophic macrophages constitutes the core of all granulomatous diseases such as tuberculosis or sarcoidosis and is decisive for disease pathogenesis. However, the molecular basis of granuloma initiation and maintenance still remain elusive. Here we showed that activation of mTORC1 in macrophages by deletion of Tsc2 was sufficient to drive macrophage hypertrophy and proliferation resulting in excessive granuloma formation in vivo. Intriguingly, mTORC1 promoted CSF1-mediated proliferation by inducing neo-expression of cyclin-dependent kinase 4 (CDK4), while simultaneously inhibiting NF-κB signaling and apoptosis. Tsc2-deficient macrophages showed constitutive CDK4 expression, hypertrophic macrophage aggregation, and proliferation in vitro that was supported by a GAPDH-mediated metabolic reprogramming towards increased glycolysis. Inhibition of mTORC1 rapidly induced apoptosis and completely resolved granulomas in myeloid Tsc2-deficient mice. Notably, in human sarcoidosis, mTORC1 activation, macrophage proliferation, and glycolysis were identified as hallmarks that correlated with a clinically progressive disease outcome. Collectively, TSC2 maintains macrophage quiescence and prevents mTORC1-dependent granulomatous disease with implications for sarcoidosis.

31 THE ROLE OF HDAC2 IN T CELLS

Preglej, T., Göschl, L. (1,2), Andersen, L. (1), Mathias, P. (3), Seiser, C. (4), Ellmeier, W. (1)

(1) Division of Immunobiology, Institute of Immunology, CePII, Medical University of Vienna, Vienna, Austria (2) Division of Rheumatology, Internal Medicine III, Medical University of Vienna, Vienna, Austria (3) Friedrich Miescher Institute for Biomedical Research, 4058 Basel, Switzerland (4) Max F. Perutz Laboratories, Vienna Biocenter, Medical University of Vienna, 1030 Vienna,

teresa.preglej@meduniwien.ac.at

The interplay of histone acetylation and deacetylation serves as a key regulatory mechanism in T cell development and function by modulating cellular gene expression. This control occurs through 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. To date, 18 individual HDACs have been identified that act in numerous cellular pathways, frequently through their repressive influence on gene transcription. Importantly, increasing evidence has implicated HDACs in participating in immunological pathways and in the regulation of T cell functions.However, the specific roles of individual HDAC family members in T cells are still subject of ongoing research. Our group could already demonstrate that HDAC1 controls the magnitude of a Th2-type inflammatory response by modulating cytokine expression in effector T cells. However, the role of the highly HDAC1-related HDAC2 protein in T cells is only poorly understood. The aim of this project is to elucidate the role of HDAC2 in T cells. Results of the ongoing study will be presented.Supported by FWF (P26193)

32 The role of mTOR signaling in macrophages for iron homeostasis

Nyamdelger Sukhbaatar (1), Monika Linke (1), Karl Katholnig (1), Stephanie Fritsch (1), Claudia Gundacker (1), Markus Hengstschläger (1), and Thomas Weichhart (1)

(1) Institute of medical genetics, Medical University of Vienna

nyamdelger.sukhbaatar@meduniwien.ac.at

33 LAMP-2 antibody sensitize endothelial cells to starvation induced cell death

Hubert, V.

Dieckmann, R. (2), Peschel, A. (3), Rees, A. (4), Kain, R. (5)

Institute of Clinical Pathology, Medical University of Vienna

virginie.hubert@meduniwien.ac.at

Pauci-immune crescentic focal necrotizing glomerulonephritis (piFNGN) is an inflammatory disease leading to kidney failure and associated with antibodies to neutrophil cytoplasmic antigens (ANCA), notably myeloperoxidase or proteinase-3. Recently autoantibodies to lysosome-associated membrane protein-2 (LAMP-2) have been shown to be highly prevalent in piFNGN, and this target is expressed on both the neutrophils and the endothelial cells. LAMP-2 antibodies are also found in ANCA-negative patient suggesting their pathogenicity although in vitro data are lacking. LAMP-2 is crucial for cellular response to stress; its deficiency increases sensitivity to cell stress in vitro. Recently we have shown that LAMP-2 antibody mimics this situation in myeloid cells by internalizing and reducing the LAMP-2 content on the lysosomes. The object of this study is to test whether antibodies are also taken up by endothelial cells and decrease their resistance to stress.Glomerular and dermal endothelial cell lines were incubated with H4B4 a mouse monoclonal antibody to LAMP-2, or with an isotype control (CD4). The results show that H4B4 is internalized by both cell lines and traffics to lysosomes via the endosomal pathway. The cell lines were then incubated for 48 hours with H4B4 or CD4 (20µg/ml) before being subjected to cell stress, namely HBSS for 6 hours in the presence of the monoclonal antibodies. Incubation with H4B4 increased the level of necrotic cell death, assessed by tryptan blue (15,90389±1,338323 % of cell death with H4B4 versus 5,189465±0,62901 with CD4) and lactate dehydrogenase release (20,641749±9,576541 % of cytotoxicity with H4B4 versus 4,123915±2,636875 with CD4). Neither H4B4 nor CD4 increased the proportion of caspase III positive cells indicating that apoptosis was unaffected. In summary, we demonstrated that LAMP-2 antibodies sensitise endothelial cells to death, and thus identified a potential pathogenic mechanism that could contribute to injury in piFNGN.

34 Resveratrol and a resveratrol-salicylate hybrid molecule: a comparative study in CD4+ T- cells

Goldhahn, K. (1); .Kloesch,B (2); Aldawsari, F. (3); Velazques-Martinez, C. (3); Steiner, G. (1,4); Schmetterer, K. (5)

Ludwig Boltzmann Cluster for Arthritis and Rehabilitation, Department for Degenerative Joint Diseases Vienna, Austria; University of Alberta, Katz Group Centre for Pharmacy and Health Research, Edmonton Alberta, CanadaMedical University Vienna, Department of Internal Medicine III, Division of Rheumatology, Vienna, AustriMedical University of Vienna, Department of Laboratory Medicine, Austria a;

katringoldhahn@gmx.at

Introduction: Aberrant T-cell responses are crucially involved in the pathogenesis of systemic autoimmune diseases such as rheumatoid arthritis (RA) leading to chronic inflammation and organ damage. Consequently, substances modulating T-cell activation may have therapeutic benefit in RA and related rheumatic diseases. Resveratrol is a natural occuring polyphenol mainly produced in plants. The beneficial effects of resveratrol are due to its anti-inflammatory, anti-carcinogenic and anti-oxidant activities. The aim of this study was to compare the effects of resveratrol and a novel resveratrol-salicylate hybrid molecule (C10) on human CD4+ T-cells.Methods: CD4+ T-cells from healthy donors were pre-incubated with different concentrations of resveratrol or C-10 before being stimulated with anti-CD3/anti-CD28 antibodies. After 24h and 72h, cell culture supernatants were harvested and IL-2, IFN-γ and TNF-α release were quantified by ELISA. Proliferation rate was measured by thymidine incorporation. In addition, the up-regulation of the early activation markers CD25, CD69, CD71 and CD98 was analyzed and phosphorylation of ERK, AKT, Stat5 and S6RP was determined by westernblot or flow cytometry. Results: Inhibition of cytokine release and proliferation rate was significantly more effective when the cells were treated with C-10. The expression of the early activation markers was reduced if the cells were exposed with resveratrol or C-10. The phosphorylation of ERK, Akt, Stat5 and S6RP was attenuated if the cells were incubated with resveratrol or C-10. Conclusion: Our data demonstrated that C-10 suppressed cytokine secretion and proliferation more effectively than resveratrol. Both compounds influence the phosphorylation of important signaling molecules. Data indicate that the resveratrol-salicylate hybrid molecule C-10 significantly amplified the effects of resveratrol in CD4+ T- cells and might be used in the future for treatment of RA.

35 Biomarkers for peanut allergy in peripheral blood derived from a whole mRNA screen in Ara h 2 specific T-cells

Saidova, A.(1), Fajgelj V.(1), Bublin M.(2), Schmidthaler K.(1),Klingelmueller F.(3), Szepfalusi Z.(1), Breiteneder H.(2), Eiwegger T.(1,4)

(1)Department of Pediatrics and Adolescent Medicine, Medical University of Vienna, (2)Department of Pathophysiology and Allergy Research, Medical University of Vienna, (3)Center for Medical Statistics, Informatics and Intelligent Systems, Medical University of Vienna, (4)Division of Immunology and Allergy, Food Allergy and Anaphylaxis Program, The Department of Paediatrics, Hospital for Sick Children,The University of Toronto

n1342711@students.meduniwien.ac.at

Background: Peanut allergy is a life-threatening IgE-mediated disease. Development of IgE-mediated responses requires the interaction of antigen presenting cells, mast cells, T cells and B cells. However, allergen specific CD4+ T cells are playing a pivotal role in the development of peanut allergy. Gene expression analysis of these allergen specific T-cell subsets may lead to a better understanding of the regulation/dysregulation of allergen specific T cells. Methods: Whole mRNA array (Agilent whole human genome oligo micro array) from allergen- specific T cells (sorted CFSE[uplow] T-cells upon exposure to Ara h 2 stimulation) from 5 peanut allergic and 6 non- peanut allergic individuals resulted in selection of 11 candidate genes (CD36, CAMK4, BMPR1a, COMMD1, TAB3, PTPN11, GIMAP8, HEMK1, ARG2, PYCRL, CSNK1E, GFPT1 and IL-13). To test for appropriateness of these genes as markers of peanut allergy in PBMCs their relative gene expression in peanut allergic (n=11), atopic poly-sensitized (n=17) and non-atopic controls (n=10) was investigated. Results: While RNA expression of TAB3 expression was significantly lower, PYCRL and HEMK1 expression was significantly higher in PBMCs of peanut allergic individuals as compared to non-allergic individuals.Conclusion: We describe three novel genes derived from an allergen specific T-cell based whole mRNA search with the potential to be used as markers for peanut allergy in peripheral blood derived mononuclear cells.Supported by the Islamic Development Bank Merit Scholarship Programme for 1433H, Saudi Arabia, the Austrian National Bank, Anniversary Fund 13846ONB, the Medical Scientific Fund of the Mayor of the City of Vienna 11013, Austrian Science Fund Project F4615-B19 and F4605-B19, Austrian Pediatric Society best Publication Award 2012.

36 Chimeric L2-based virus-like particle (VLP) vaccines targeting cutaneous human papilloma viruses (HPV)

Huber, B. (1), Schellenbacher, C. (1), Shafti-Keramat, S. (1), Kirnbauer, R. (1)

(1) Laboratory of Viral Oncology (LVO), DIAID, Department of Dermatology, Medical University of Vienna

bettina.huber@meduniwien.ac.at

Common cutaneous HPV induce skin, palmo-plantar and plane warts (HPV1/2/4/27/57; 3/10). Species beta HPV (HPV5, 8, etc) are hypothesized to play an adjunct role (besides the main carcinogen UV-light) in non-melanoma skin cancer development in immunosuppressed patients. Licensed 2- and 4-valent HPV vaccines contain major capsid protein L1 VLP that target HPV6/11 causing 90% of genital warts, and/or HPV16/18 causing a majority of cervical carcinomas, and a fraction of other ano-genital and oro-pharyngeal cancers. They do not however protect against any of the cutaneous types, as L1 VLP vaccination induces a largely type-restricted protective immune response. To broaden the spectrum of current HPV vaccines, chimeric VLP were designed that are based upon the minor capsid protein L2 that contains type-

common cross-neutralization epitopes. Amino (N)-terminal HPV16 L2 epitopes amino acid (aa) 14-33 (“RG1”) and aa53-72 have been identified previously to induce cross-neutralizing antisera. Analogous peptides of the highly conserved regions from cutaneous types HPV17 (RG1) and HPV5 (aa53-72) were inserted into L1 surface loops of heterologous types HPV5/16/18. Chimeric fusion proteins were expressed in Sf9 insect cells by recombinant baculoviruses and were able to assemble into VLP and to elicit a humoral response against the inserted epitope, as assessed by ELISA. Immunization of rabbits with alum-MPL adjuvanted chimeric VLP displaying the RG1-epitope, but not VLP with insertions of the aa53-72-homologous epitope, induced cross-neutralizing antisera in vitro by pseudovirion (PsV) neutralization assays. In vivo, passive transfer of 5L1-17RG1 or 16L1-17RG1 VLP-raised sera (cross-)protected against HPV20, or HPV5/20/96/16, respectively, in a murine challenge model. In conclusion, chimeric L1-L2 based VLP are promising vaccine candidates to induce cross-protection against the plethora of clinically relevant cutaneous types, albeit choice of the L2 epitope appears crucial.

37 How well does the Framingham Prediction Model predict the Cardiovascular Risk of Austrians?

Wallisch, C. (1) Mundigler, G. (2), Winkelmayer, W. C. (3), Heinze, G. (1), Dunkler, D. (1)

(1) Medical University of Vienna, CeMSIIS, Section for Clinical Biometrics, Vienna, Austria, (2) Medical University of Vienna, Department of Medicine 2, Division of Cardiology, Vienna, Austria, (3) Baylor College of Medicine, Section of Nephrology, Houston, TX, USA

christine.wallisch@meduniwien.ac.at

Introduction: Austria’s health screening program (AHSP) is designed to improve prevention of various diseases including cardiovascular disease (CVD) — a leading cause of death and disability. The Framingham CVD model is a popular CVD risk prediction tool, but used an American study cohort. Thus we investigated how well it predicts CVD risk in an Austrian cohort consisting of AHSP participants. Methods: Our validation cohort comprised all 1.4M AHSP participants in 2009-2015 aged 30-74 years (52% women). CVD events were defined by a CV cause of hospitalization or death.Framingham models for women and men for a prediction horizon of 5 years were evaluated by assessing a) discrimination, the ability of prediction models to distinguish individuals with different outcomes, using c-indices, and b) calibration, measuring the agreement between observed and predicted risk. Furthermore, we re-estimated the model coefficients with the Austrian data and computed the proportion of variability in the outcome that the models explained (PVE). Results: In women and men, we observed 0.82 and 1.36 CVD events per 100 years of observation time, respectively. Calibration plots revealed that the Framingham models overestimated CVD risk in the Austrian population. While for the original derivation cohort c-indices of 0.79 in women and 0.76 in men were reported, the Framingham models attained values of only 0.66 and 0.67 at validation, which increased to 0.69 and 0.72 after

re-estimation, respectively. PVEs by the re-estimated models were 3.12% and 5.32%. Decomposing PVE revealed that age was the most important predictor (1.26% and 3.02%) followed by treated or untreated systolic blood pressure (0.20% and 0.25%).ConclusionSex-specific Framingham CVD prediction models performed poorly in the Austrian preventive screening population. Therefore, we propose recalibration, re-estimation or even re-development of a model suitably predicting CVD risk in the Austrian general population.

38 CLINICAL SIGNIFICANCE OF THE SINGLE NUCLEOTIDE POLYMORPHISM TLR2 R753Q IN HEART TRANSPLANT RECIPIENTS AT RISK FOR CYTOMEGALOVIRUS DISEASE

Martina Schneider (1), Teresa Matiqi (1), Michael Kundi (2), Franz JJ Rieder (1), Martin Andreas (3) , Robert Strassl (4) , Andreas Zuckermann (3) , Christof Jungbauer (5) , Christoph Steininger (1)

(1) Department of Medicine I, Div. of Infectious Diseases and Tropical Medicine, Medical University of Vienna, (2) Institutes of Environmental Health, Center for Public Health, Medical University of Vienna, (3) Department of Surgery, Div. of Cardiac Surgery, Medical University of Vienna, (4) Department of Laboratory Medicines, Division of Clinical Virology, Medical University of Vienna, Vienna, Austria (5) Blood Service for Vienna, Lower Austria and Burgenland, Austrian Red Cross, Vienna, Austria

martina.a.schneider@meduniwien.ac.at

Background: The Toll-like receptor 2 (TLR2) is a significant component of innate immunity against cytomegalovirus (CMV) infection but information on the clinical significance of the TLR2 single nucleotide polymorphism (SNP) R753Q (rs5743708) is conflicting. Methods: The presence of the TLR2 polymorphism was determined by a genotyping assay of 175 Heart Transplant Recipients (HTX) patients and 281 healthy blood donors and evaluated in relation to selected virological and clinical parameters. Results: Relative frequency of TLR2 polymorphism was similar in HTX patients and blood donors (homozygous wild-type, 94.3% vs. 94.0%; heterozygous, 5.1% vs. 5.7%; homozygous mutated, <1%). CMV viremia was detectable in 108 (61.7%) of HTX patients. The TLR2 polymorphism was neither associated with occurrence or level of CMV infection nor with survival, graft failure or rejection, or CMV serostatus of patient before transplantation. Nevertheless, CMV viremia occurred in 83.1% of R+/D+, 77.1% of R+/D-, and 64.3% of R-/D+ patients. Time of first CMV viremia was in R-/D+ patients later than in CMV-seropositive patients (median, 182 days versus 23 days; P<0.001) corresponding to the duration of antiviral prophylaxis in R-/D+ patients.Conclusion: This study suggests that the TLR2 R753Q polymorphism is extremely rare in the general population and HTX patients and therefore other prophylactic strategies such as CMV infection status and use of antiviral compounds is more efficient than screening for this polymorphism.

39 Transmembrane domains of the tick-borne encephalitis virus E protein in virus assembly

Blazevic, Janja, Heinz, F. X., Stiasny, K.

Department of Virology

janja.blazevic@meduniwien.ac.at

1. Introduction The major surface protein E of flaviviruses possesses a peculiar double membrane anchor, consisting of two antiparallel transmembrane domains (TMDs) which execute important functions in the biosynthesis and processing of the viral polyprotein. 2. ObjectivesIn the course of this project, we wanted to find out whether interactions within and/or between the double membrane anchors of E are involved in the assembly processes of one of the major human pathogenic flaviviruses, tick-borne-encephalitis virus (TBEV). This virus is closely related to the mosquito-borne yellow fever, dengue, Japanese encephalitis, and West Nile viruses. 3. Materials & MethodsUsing the infectious clone of TBEV we have replaced the E TM regions by the homologous elements of the related Japanese encephalitis virus (JEV) in different combinations. This approach allows the investigation of TMD interactions required for flavivirus assembly without affecting polyprotein processing. 4. ResultsThe virus mutant containing the complete heterologous JEV E membrane anchor was impaired in its ability to release infectious virus particles suggesting that interactions with other viral proteins are required for efficient virus assembly. An even stronger reduction of virus production was observed for the mutants containing mixed TBEV-JEV E membrane anchors indicating a requirement for homologous TMD interactions. To identify such interactions more precisely, serial passaging experiments with the mutants will be performed to allow the viruses to acquire compensatory mutations.5. ConclusionOur data provided evidence that TMD interactions are required for efficient assembly and release of infectious virus particles.

40 Allergen-induced relapse of allergic asthma by long-lived resident CD4+ Th2 memory cells

Kazemi,S.(1), Bošnjak, B.(1), Altenburger, L.M.(2), Mokrović, G.(3), Epstein, M.M.(4)

Department of Dermatology, DIAID, Experimental Allergy Lab, Medical University of Vienna, Austria

n1242832@students.meduniwien.ac.at

Background: Allergic asthma is a chronic disease characterized by lung inflammation and allergen-induced exacerbations. Despite the wide availability of effective treatments, severe asthma remains a challenge. We sought to understand the mechanisms underlying allergen-specific disease relapses in a model of relapsing-remitting nature of allergic asthma in mice.Methods: Female BALB/c mice from 139 to 636 days recovered from the initiation of allergic asthma, were injected with fluorescently-labeled anti-CD4 mAb (RM4-4) intravenously to label CD4+ T cells and 10 minutes later cells from lungs and spleen were phenotyped and functionally evaluated. To further characterize allergen-specific CD4+ T helper (Th) cells and their effect during disease relapse, we injected a depleting anti-CD4 (GK1.5) mAb or fingolimod, FTY720 for helper T cell depletion or inhibition of cell migration, respectively. Results: Long-lived Th2 memory CD4+ T cells residing in persistent lung inflammatory infiltrates of mice recovered from the initiation of disease immediately responded to secondary allergen challenge by inducing allergic lung and airway inflammation, mucus hypersecretion and increased allergen-specific IgE and resisted CD4 depletion and FTY720 treatment. Further phenotypic and functional evaluation ex vivo revealed that these cells are allergen-specific 'resident' Th2 memory cells. Conclusions: Our data demonstrate that incalcitrant pathogenic allergen-specific long-lived resident Th2 memory cells cause allergic asthma relapses and provide an important potential target for therapeutic intervention.

41 Flavivirus E protein stem interactions in virus entry

Medits, I (1) Heinz, F.X. (1), Stiasny, K. (1)

(1) Department for Virology, Medical Univerity of Vienna

iris.medits@meduniwien.ac.at

Flaviviruses enter cells by receptor-mediated endocytosis. After virus uptake, the viral envelope protein E mediates fusion of the viral and the endosomal membrane, triggered by the slightly acidic pH in endosomes. 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.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.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.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.

42 Flavivirus binding to cells

Haslwanter, D. (1) Blaas, D. (2), Blazevic, J. (1), Heinz, F.X. (1), Stiasny, K. (1)

Medical University of Vienna, Max F. Perutz Laboratories, Department of Medical Biochemistry, Vienna, Austria

denise.haslwanter@meduniwien.ac.at

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. 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 cells from different host species and several attachment factors have been identified, but a high affinity receptor has not been described yet.In this study, we want to investigate the binding of tick-borne encephalitis virus (TBEV) and other flaviviruses 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.We used purified TBEV preparations and various cell types for our study. Bound and unbound virus was quantified by two-step RT qPCR and infectivity was measured by focus forming assays. Human rhinovirus 2 (HRV2), with a known high-affinity receptor, served as a control.In contrast to HRV2, TBEV bound very inefficiently to the different cell types used, with less than 1% 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 different cell types. Moreover, we determined the infectivity of unbound virus particles by repetitive transferring a virus preparation to new cells. After 10 rounds of cell binding, infection was not strongly decreased indicating an underestimation of the actual number of infectious particles by conventional infectivity assays.Currently we investigate factors that underlie these phenomena. In addition, we will analyze different conditions that could potentially enhance binding of TBEV to cells and will compare this to other flaviviruses.

43 Polyamines control proliferation and survival of macrophages in an mTORC1-dependent manner

Fritsch S.D. Magnes C., Weichhart T., Demel F., Weckwerth W., Hengstschläger M.

Medizinische Universität Wien

stephanie.fritsch@meduniwien.ac.at

The mammalian target of rapamycin (mTOR) has a central role in the effector functions of innate immune cells, as it couples cellular activation to the environmental and intracellular energy status. Polyamines (PAs) such as ornithine, spermidine, and spermine are essential for normal cell growth and development. However, elevated levels are associated with various pathologies, especially cancer. Furthermore, they are involved in M2 polarisation of macrophages (MΦ). In our previously described mouse model, we constitutively activated the mTOR complex 1 (mTORC1) specifically in myeloid cells by deletion of its negative regulator TSC2 via the Cre/LoxP system (TSC2ΔM). We observed an expansion of strongly proliferative M2-like MΦ in various tissues. Moreover, a shot-gun metabolomic analysis of the lung indicated that the PA synthesis pathway is affected in TSC2ΔM mice. Therefore, we analysed PA levels in bone marrow-derived macrophages (BMDM) and found them significantly enriched in TSC2ΔM mice. Moreover, the mTOR inhibitors rapamycin and Torin1 suppressed expression of these PAs in TSC2ΔM BMDM, demonstrating that activation of mTORC1 promotes polyamine synthesis in MΦ. Molecularly, we identified the antizyme inhibitor 1 (AZIN1), which is important for maintaining intracellular PA levels, as target of mTORC1. qPCR and western blot data showed increased levels of AZIN1 in TSC2ΔM BMDM that was dependent on rapamycin. Further analysis revealed additional mTORC1-dependent enzymes of PA metabolism. Functional evaluation showed that the highly increased proliferative capacity and elevated viability of TSC2ΔM MΦ was decreased by treatment with different PA inhibitors (DFMO and DENSPM). These data demonstrate that TSC2 and mTORC1 control the synthesis of PAs to regulate proliferation and survival in MΦ. In future studies, we want to investigate the role of mTOR-dependent PA synthesis in MΦ for various pathologies such as granuloma formation and cancer.

44 Type I interferons promote the intracellular replication of the human fungal pathogen Candida glabrata in murine macrophages

Riedelberger Michael (1), Kuchler Karl (1)

(1) Max F. Perutz Laboratories, Medical University Vienna, Department of Medical Biochemistry, Campus Vienna Biocenter, Austria

michael.riedelberger@meduniwien.ac.at

During infections with various pathogens, the family of type I interferons (IFNs) engage a predominant role in the complex regulation of the antimicrobial immune response and the

molecular cross-talk between immune cell populations. However, the contribution and molecular function of type I IFNs remains poorly understood or even controversial for infections with bacteria or fungi.Here, we show that type I IFNs promote the survival of the human fungal pathogen Candida glabrata during host-pathogen interactions by increasing the intracellular replication of C. glabrata in murine macrophages. In detail, type I IFNs suppress the transcription of host genes involved in iron metabolism and, thus, possibly shift the intracellular iron content to a more favourable environmental niche for C. glabrata. In line with this, elevated iron levels in macrophages support the intracellular replication of C. glabrata, which can be suppressed by iron chelation. Consistently, the beneficial type I IFN-mediated effects on C. glabrata replication can be reverted by intracellular chelation of iron within macrophages. Therefore, our results suggest a detrimental role of type I IFNs for the host during C. glabrata infections by regulating the cellular iron homeostasis in macrophages.

45 ANTI-PROSTATE CANCER ACTIVITY OF THE CHINESE HERBAL MEDICINE PANAX QUINQUEFOLIUS SAPONIN

He, S. (1), Lou, L. (2), Liu, L. (3), Wang-Mattes, S. (1), Li, S. (4), Ma, Y. (1)

(1) Department of Pathophysiology and Allergy Research, Center of Pathophysiology, Infectiology & Immunology, Medical University of Vienna, Vienna, Austria. (2) Xiyuan Hospital, China Academy of Chinese Medical Sciences, Beijing, China. (3) Beijing Hospital of Traditional Chinese Medicine, Capital Medical University, Beijing, China. (4) Department of Pharmaceutical Analysis and Metabolomics, Jiangsu Province Academy of Traditional Chinese Medicine and Jiangsu Branch of China Academy of Chinese Medical Sciences, Nanjing, China

shan.he@meduniwien.ac.at

Prostate carcinoma is the most commonly diagnosed cancer and the second most common cause of cancer mortality among men in the USA and in Europe. The major causes of the disease remain unknown, although hormonal status and lifestyle are known risk factors. Hormone therapy, in the form of androgen ablation therapy, is an effective treatment during the early stages of prostate cancer. However, during hormone therapy, 80-85% of prostate cancers progress to an androgen-independent type of prostate cancer, known as recurrent androgen-independent prostate cancer (AIPC). AIPC is a lethal form of prostate cancer and there is currently no effective therapy for it. Therefore, there is a significant need for the development of new therapeutic approaches for the treatment of prostate cancer. The Chinese herbal medicine Panax quinquefolius has been tested for its tumor selectivity and cytotoxic efficacy. P. quinquefolius saponins (PQS) have been shown to have anti-tumor effects. The aim of this study is to evaluate the anti-cancer activity of PQS in human prostate cancer cells. The human prostate cancer cells DU145 were treated with PQS for 24h, 48h and 72h. The cell viability assay and FACS based apoptosis assay were performed after treatment. Action mechanism was determined

by invasion assay and wound healing assay. The inhibitory effects on the proliferation of the human prostate cancer cells indicated that PQS inhibits the growth of prostate cancer cells. RT-PCR experiments and western analysis demonstrated that PQS treatment regulated the expression of p53, TMEM79 and bcl2. Invasion assay and wound healing assay showed that a significant decrease in cell invasion and migration after PQS treatment. In conclusion, PQS promotes prostate cancer cells apoptosis and inhibits the proliferation of prostate cancer cells suggests that PQS might be an effective herbal remedy for treating prostate cancer.Supported by the BMWF/BMG (GZ 402.000/0006II/6b/2012).

46 An interdisciplinary approach to identify novel causal genes in rare diseases

Julia Pazmandi(1) Jörg Menche(1), Kaan Boztug (2)(3)(4)

(1)CeMM Research Centre of Molecular Medicine of the Austrian Academy of Sciences, (2)Associate Professor, Dept. of Pediatrics and Adolescent Medicine, Medical University of Vienna, (3)Director, Vienna Center for Rare und Undiagnosed Diseases (CeRUD)

jpazmandi@cemm.oeaw.ac.at

Aim: Identification of causative genes in rare diseases offers unprecedented opportunity to understand biological mechanisms and pathways. However, it also poses significant challenges. Research often involves data retrieved from small pedigrees with single patients presenting heterogeneous phenotypes. Even after the introduction of high throughput genetic screening by whole exome sequencing (WES), in many cases several candidate genes remain to be investigated and validated to unambiguously identify the disease-causing variants, making the process highly time, cost and labor intensive. Therefore, in rare diseases an intelligent and sophisticated candidate prioritization is needed. We are here conceptualizing a bioinformatics-based pathogenicity prediction coupled with systems biology approach in early-onset inflammatory bowel disease (EOIBD) as a model disease, to gain a comprehensive view over the relevant signaling axis in EOIBD and show feasibility of such an approach in different subtypes of rare diseases. Method: We propose the construction of the IBDOME; a unifying weighted disease network enriched for immune specific biological functions, disease specific gene expression data, and IBD specific pathways and molecular functions using IBD-associated seed genes. Network exploration strategies such as diffusion algorithms, key driver analysis and Bayesian network propagation will provide key information on affected disease gene neighborhoods and clusters. Using the IBDOME with variant pathogenicity prediction in a synergistic manner could significantly improve causal variant prediction of individual patients. Results: We propose an interdisciplinary variant prediction method that will combine genomics, proteomics, and network biology. We hypothesize that this integrative approach will be more successful in identifying disease drivers than evaluating single candidates, and may be feasible to apply to other groups of rare diseases as well.

47 Human Cytomegalovirus Phosphoproteins are Hypophosphorylated and Intrinsically Disordered

Rieder F. J. J. (1) Kastner M-T. (1), Hartl M. (2), Puchinger M.G. (3), Schneider M. (1), Majdic O. (4), Britt W.J. (5), Djinović-Carugo K. (3,6), Steininger C. (1)

(1) Department of Medicine I, Division of Infectious Diseases and Tropical Medicine, Medical University of Vienna, Vienna, Austria (2) Mass Spectrometry Facility, Max F. Perutz Laboratories (MFPL), Vienna Biocenter (VBC), Vienna, Austria (3) Department of Structural and Computational Biology, Max F. Perutz Laboratories, University of Vienna, Vienna, Austria (4) Center for Pathophysiology, Infectiology and Immunology, Institute of Immunology, Medical University of Vienna, Vienna, Austria (5) Department of Pediatrics, Children's Hospital, University of Alabama at Birmingham, Birmingham, Alabama, USA (6) Department of Biochemistry, Faculty of Chemistry and Chemical Technology, University of Ljubljana, Ljubljana, Slovenia

franz.rieder@meduniwien.ac.at

Phosphorylation of proteins has important regulatory functions in cell homeostasis and is tightly regulated by kinases and phosphatases. The tegument of human cytomegalovirus (CMV) comprises several proteins that are reported to be extensively phosphorylated but also of two cellular protein phosphatases (PP1 & PP2A). To investigate this apparent conflict, we evaluated the phosphorylation status of the tegument proteins pUL32 and pp65 by enzymatic dephosphorylation and mass spectrometry. Enzymatic dephosphorylation with bacterial λ phosphatase, but not with PP1, shifted the pUL32-specific signal on reducing SDS-PAGE from 150 kDa to 148 kDa – still much larger than the ~118kDa obtained from our diffusion studies and from the calculated protein mass of ~113 kDa. Remarkably, inhibition of phosphatases by treatment with the phosphatase inhibitors Calyculin A and Okadaic acid resulted in a shift to ~200 kDa or ~180, respectively, indicating that a considerable number of potential phosphorylated residues on pUL32 are not phosphorylated under normal conditions. Mass spectrometry revealed a general state of hypophosphorylation of CMV phosphoproteins with only 17 phosphorylated residues detected on pUL32 and 19 on pp65, respectively. Moreover, we found in silico evidence that the C-terminal two-thirds of pUL32 are intrinsically disordered and that most phosphorylations mapped to this region. In conclusion, we show that important CMV tegument proteins are indeed phosphorylated though to a lesser extent than previously reported and the difference in migration velocity and calculated mass of pUL32 may not be attributed to phosphorylation but more likely be due to the partially intrinsically disordered nature of pUL32.

48 Determining the influence of cell adhesion and costimulation on T-cell antigen recognition

Doel-Perez, I. (1), Brameshuber, M.(2), Platzer, R.(1), Kellner, F. (1), Huppa J.B. (1)

Medical University of Vienna, Technical University of Vienna

CePII, Medical University of Vienna

iago.doelperez@meduniwien.ac.at

48A Prevention and Therapy of Allergy by Toxoplasma gondii- and Oesophagostomum dentatum-derived Immunomodulatory Molecules

Mirjana Drinić[1], Irma Schabussova[1], Angelika Wagner[1], Bärbel Ruttkowski[2], Anja Joachim[2], Ursula Wiedermann[1]

[1]Institute of Specific Prophylaxis and Tropical Medicine, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Austria

[2] University of Veterinary Medicine, Vienna, Austria

mirjana.drinic@meduniwien.ac.at

The link between reduced incidence of allergic diseases and infection with certain parasites has been repeatedly confirmed in numerous epidemiological and experimental 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 both infection and treatment with crude extracts of Toxoplasma gondii and Oesophagostomum dentatum prevented allergic immune responses and airway inflammation in a mouse model of type I allergy. Preliminary results show that upon heat-inactivation the suppressive effect of O.dentatum is stabile, whereas T. gondii extract loses its immunomodulatory potential. Therefore, our aim is to identify and characterize fractions of these parasites with immunomodulatory properties and to possibly isolate the respective parasitic molecules. Currently we are focusing on identifying which of the molecular groups are responsible for the immunomodulatory effect. Therefore, the extracts were deglycosylated by means of metaperiodate oxidation or enzymatic treatment with PNGase F, digested with proteinase K or subjected to chloroform/methanol lipid extraction in order to obtain fractions with inactive glycans, proteins or lipids, respectively. These fractions will be used for in vitro analysis of the intrinsic immunomodulatory properties in comparison to whole extracts by stimulating splenocytes and DCs and measuring the cytokine production. Identified fraction will then be additionally characterized with different techniques such as HPLC, 2D gel electrophoresis, followed by MALDI-TOF-MS and ESI-MS/MS in order to identify specific

molecule with immunomodulatory/anti-allergic properties, which will then be purified/produced and tested in vitro and in vivo. The ultimate goal is the use of parasite derived adjuvants with immunosuppressive effects in future allergy vaccines.

48B Quantitative assessment of B-cell subsets: From digital pathology to disease mechanisms attributed to germinal centers

Mungenast, F.1, Meshcheryakova, A.1, Oswald, A.1, Bajna, E.1, Tamandl, D.2, Bergmann, M.3, Koperek, O.4, Birner, P.4, Mechtcheriakova, D.1

1Department of Pathophysiology and Allergy Research, Center of Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria

2Department of Radiology and Nuclear Medicine, Medical University of Vienna, Vienna, Austria

3Department of Surgery, Medical University of Vienna, Vienna, Austria

4Division of Clinical Pathology, Department of Pathology, Medical University of Vienna, Vienna, Austria

felicitas.mungenast@meduniwien.ac.at

Despite accumulated knowledge positioning tumor-infiltrating B cells among powerful contributors to tumor immunity, many questions remain given complexity of multifarious B-cell subsets and unique ability to assemble into functional ectopic follicular structures. Recently, our group demonstrated that $CD20^+$ B cells organized into follicular structures at the metastatic site of patients with colorectal cancer (CRC) are strongly associated with a better prognosis. However the mechanisms for development and function of follicular structures within diseased tissues as well as at physiological conditions within secondary lymphoid organs are not clearly understood yet; limited knowledge is available on the magnitudes of post-germinal memory and/or plasma cell subsets.

We developed a computerized microscopy-based algorithm, using TissueFAXS platform, allowing quantitative assessment of memory and plasma B cells across large-scale paraffin-embedded tissue specimens. 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 $CD138^+/CD27^{high}$ plasma cells.

We first assessed the distribution and quantities of B-cell subsets in different pre-defined compartments of classical follicles (germinal center, mantle zone, surrounding 100 µm rim) within the tonsil tissues. Within the ongoing study, we applied the established strategy to characterize B-cell aggregates and ectopic follicular structures formed within primary CRC tissue and matched metastatic CRC in the liver.

The results of the pilot study indicate (i) the tumor anatomy-attributed and patient-specific distribution and organization patterns of B cells including functionally active ectopic follicular

structures at both primary and metastatic CRC sites and (ii) the presence of various B-cell memory subsets.

Supported by FWF P22441-B13/P23228-B19

49 Effects of Imiquimod on hair follicle stem cells and hair cycle progression

Amberg, N. (1) Holcmann, M. (1), Stulnig, G. (1), Sibilia, M. (1)

Institute of Cancer Research, Medical University of Vienna, Borschkegasse 8a, 1090 Vienna

nicole.amberg@meduniwien.ac.at

Topical Imiquimod application is widely used as a model for psoriasiform-like skin inflammation in mice. Because laboratories employ different experimental conditions using Imiquimod, it is unclear how the hair cycle stage influence the experimental outcome. Here we investigated how Imiquimod affects hair follicle stem cells and cycling, and whether the timing of Imiquimod application influences the immune infiltrate. Our results show that Imiqumod application at mid and late Telogen activated hair follicle stem cells leading to premature hair cycle entry (Anagen), which was accompanied by massive infiltration of inflammatory cells like macrophages, neutrophils, monocytes and mast cells. Interestingly in Rag-/- mice, which harbored increased macrophage numbers in the skin, Anagen induction following IMQ treatment was significantly reduced but could be restored after macrophage depletion suggesting that T cells were not primarily required for hair follicle activation by IMQ. Based on our findings we recommend conducting experiments with topical Imiquimod on razor shaved mice during mid and late Telogen as the biggest differences in immune cell composition can be seen after Imiquimod treatment. Moreover, we provide evidence that hair follicle stem cell activation after Imiquimod treatmtent is mediated by macrophages.

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

Stulnig, G.(1) Drobits, B.(1), Novoszel, P.(1), Holcmann, M.(1), Sibilia M.(1)

(1) Institute of Cancer Research, Medical University of Vienna, Vienna, Austria

gabriel.stulnig@meduniwien.ac.at

Imiquimod (Imi) is an agonist of toll like receptor 7/8 (TLR7/8), a pathogen recognition receptor that recognizes single stranded RNA. Imi exerts therapeutic anti-viral and anti-tumor effects in both mice and humans. Therapeutically, Imi is applied topically as a 5% cream formulation under the trademark Aldara. Previously, our group showed that Imi treatment leads to tumor clearance in a mouse model of melanoma. We showed that the anti-tumor effect of Imi is accompanied, among others, by the accumulation of plasmacytoid dendritic cells (pDCs). We could furthermore show that Imi activated pDCs acquire tumor killing effector properties by upregulating the cytolytic molecules TRAIL and granzyme B. By employing a transgenic mouse model to specifically deplete pDCs, we demonstrated that pDCs are crucial for the tumoricidal properties of Imi. In search for the molecular pathways conferring tumor-killing activities to Imi-stimulated pDCs, we found that pDC infiltration to Imi treated skin requires the chemokine CCL2. Thus, current studies are addressing the anti-tumor efficacy of Imi in CCL2-/- mice. Albeit the important effects of Imi in tumor immune biology, we and others have shown that repeated topical application of Imi on murine skin leads to skin inflammation and is used as an established mouse model of psoriasiform dermatitis. While addressing the function of pDCs in this process, we found that pDCs exert regulatory properties during Imi induced skin inflammation. Current studies are aimed at elucidating the mechanism by which pDCs modulate the severity of Imi mediated skin inflammation.

51 A PAK2-STAT5 axis is key for tumor formation of BCR-ABL+ cells in vivo

Berger-Bečvař, A. (1), Edlinger, L. (1), Hoelbl-Kovacic, A. (1), Greiner, G. (2), Hoermann, G. (2), Sexl, V. (1)

(1) Institute of Pharmacology and Toxicology, University of Veterinary Medicine Vienna, (2) Department of Laboratory Medicine, Medical University of Vienna

leo.edlinger@vetmeduni.ac.at

Introduction: STAT5 is an essential transcription factor required for disease initiation and disease progression by BCR-ABL oncogenes. STAT5 regulates survival, proliferation, and therapeutic responses in chronic myeloid leukemia (CML) and B acute lymphoblastic leukemia (B-ALL). We could previously show that – beside the well described tyrosine phosphorylation site on Y699 – a mutation of a serine to alanine (S779A) significantly increases diseases latency in BCR-ABL-mediated B-ALL showing the importance of this phosphorylation site. Subsequently, we identified PAK1 and PAK2 as upstream kinases of S779 phosphorylation. Therefore, we here aimed to investigate the role and potential differences between PAK1 and PAK2 in BCR-ABL+ cells.Materials and methods: Knockdown of PAK1 and/or PAK2 was performed in human BCR-ABL+ cells, and differences in cell cycle and growth were assessed in vitro. In addition, we injected the cells subcutaneously into immunocompromised mice and monitored tumor growth.Results: No significant differences in cell cycle characteristics were found in vitro upon

single knockdown of PAK1 or PAK2, whereas knockdown of both PAK1 and PAK2 was incompatible with cell survival. Interestingly, tumor volume and tumor weight were drastically reduced when PAK2 was knocked down while PAK1 knockdown had no effect in vivo.Conclusions: While PAK1 and PAK2 compensate each other for growth and survival of BCR-ABL+ cells in vitro, only PAK2 is required for survival and growth in vivo.

52 The role of the protocadherin CDHR5 in colorectal cancer

Awad, M.

Krieger, S. (2), Milovanovic, D. (3), Kenner, L. (4), Kerjaschki, D. (5), Eferl, R. (6)

Medical University Vienna

monira.awad@meduniwien.ac.at

Protocadherins constitute the largest subgroup of the cadherin protein superfamily and are frequently downregulated in human cancers suggesting a negative role in oncogenesis. The protocadherin CDHR5 (Mucin-Like Protocadherin) is a transmembrane protein that is located in the microvillar brush border of enterocytes, cholangiocytes and kidney epithelial cells. CDHR5 crosslinks microvilli and has been implicated in regulation of [beta]-Catenin activity. We are interested in CDHR5 functions in colorectal cancer. We found that CDHR5 expression is downregulated in altered crypt foci, adenomas, carcinomas and colorectal liver metastasis. We further demonstrate a tumor-suppressive role of CDHR5 in colorectal cancer using transplantation experiments of cell lines with gain or loss of CDHR5 function. We generated CDHR5 knock-out mice to further investigate CDHR5 functions in autochthonous colorectal tumors. Knock-outs were viable and did not show an overt intestinal phenotype but displayed shortening of microvillus length. Formation of colorectal cancer, induced with the chemical Azoxymethane/Dextransulfate protocol, was not affected in CDHR5 knock-out mice but the number of aggressive carcinomas invading the muscularis mucosa was substantially increased. These data suggest that CDHR5 is a metastasis suppressor gene in colorectal cancer. We are currently using intestinal organoid cultures, cotransfection experiments and RNASeq of RNA, isolated from intestinal epithelial cells, to unravel molecular function of CDHR5 in colon cancer metastasis.

53 The Role of CD40/CD40L pathway in the field of transplantation

Granofszky, N. (1), Hock, K.(2), Mahr, B(3), Pilat-Michalek, N. (4), Wekerle, T. (last)

Medical University of Vienna

nicolas.granofszky@meduniwien.ac.at

Background: Several T-cell costimulatory pathways have been identified to play a critical role in promoting transplant rejection, but their function on distinct T cell subsets remains incompletely defined. Therefore the goal of our research is to investigate the mechanistic role of CD40 and CD40L in specific T cell subsets, in particular in conventional T cells versus regulatory T cells. Methods: Protein kinetic studies of CD40 and CD40L with anti-CD3/CD28 stimulated C57BL/6 splenocytes were performed. CD40+/- and CD40L+/- T cells were further analyzed by FACS for their different expression behavior of selected. CD40 KO mices were used for allogeneic skingraft rejection experiments and the role of effector/memory (CD62Llow/CD44high) subsets within this model was followed over time.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. Notably, 40-50% of Treg cells expressed CD40L. While Helios and CD62L were expressed more frequently within FoxP3+ CD40L negative cells, ICOS expression was higher on CD40L+ (36.4%) than CD40L- (28%). Only a small subset of T-cells (~1%) expressed CD40 and within those around 12-13% were single positive for CCR6. Skingraft experiments revealed that in the absence of CD40 in both donor and recipient, graft survival was only prolonged for additional 3 days. Conclusion: This preliminary study reveals a time and stimulus-dependent induction of CD40L on distinct T-cell subsets, including FoxP3 Tregs. CD40 is low and transient expressed on T-cells, however CD40+ population exhibit a stronger CCR6 expression which can be associated with a stronger capability for migration to inflammatory sites during immunological processes like graft rejection. The lack of CD40 in donor and recipients is associated with better skingraft survival but a disrupted memory Tcells development.

54 The role of nuclear receptor corepressor 1 (Ncor1) in peripheral T cells

Hainberger, D. (1) ,Müller, L. (1), Auwerx, J. (2), Ellmeier, W. (1)

(1) Division of Immunobiology, Institute of Immunology, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria, (2) Laboratory for Integrative and Systems Physiology, École polytechnique fédérale de Lausanne, Lausanne, Switzerland

daniela.hainberger@meduniwien.ac.at

Nuclear receptor corepressor 1 (Ncor1) has been originally identified as a corepressor of nuclear receptor-mediated gene repression. The repressive activity of Ncor1, and its homologue Smrt (Ncor2), is mediated via recruitment of chromatin complexes that include chromatin modifying enzymes such as histone deacetylases (HDACs). Studies with Ncor1 knockout mice (which are embryonic lethal) revealed important functions for Ncor1 during early embryonic development,

such as neural cell differentiation, erythropoiesis and a block at the DN stage in developing fetal thymocytes, highlighting its essential role for development and differentiation. Beyond transcription factors of the nuclear receptor family, Ncor1 interacts also with several members of the BTB zinc finger (BTB-ZF) transcription factor family such as PLZF, BCL6 and MAZR, which are key regulators of T cell development and function. Together, this implies important roles for Ncor1 in T cells. To test this hypothesis, we have generated mice with a T cell-specific deletion of Ncor1 (using Cd4Cre). Preliminary results indicating altered T cell activation and cytokine production will be presented.

55 The role of STAT1 in colitis-associated colorectal cancer

Crncec, I.(1), Gordziel, C.(2), Svinka, J.(1), Pathria, P.(3), Modak, M.(4), Stöckl, J.(4), Schlederer, M.(5,6), Kenner, L.(5,6), Müller, M.(7), Strobl, B.(7), Friedrich, K.(2), Eferl R.(1)

(1) Medical University Vienna & Comprehensive Cancer Center (CCC), Institute for Cancer Research, Vienna, Austria, (2) University Hospital Jena, Institute of Biochemistry II, Jena, Germany, (3) Moffitt Cancer Center, Tampa, Florida USA, (4) Institute of Immunology, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria, (5) Ludwig Boltzmann Institute for Cancer Research LBICR, Vienna, Austria, (6) Institute of Clinical Pathology, Medical University of Vienna, Austria, (7) Institute of Animal Breeding and Genetics, University of Veterinary Medicine Vienna, Austria

ilija.crncec@meduniwien.ac.at

STAT1 is an interferon-inducible transcription factor that acts as a tumor suppressor in a number of malignancies due to its ability to integrate anti-proliferative and pro-apoptotic functions of IFN as well as elicit an immune response against tumor cells. We investigated the role of STAT1 in tumorigenesis by applying the Azoxymethane/Dextrane Sulfate Sodium (AOM/DSS) protocol to mice with an intestinal epithelial cell specific deletion of the Stat1 gene (Stat1up[[delta]IEC]). The application of this protocol resulted in an increased tumor load and tumor multiplicity in male Stat1up[[delta]IEC] mice only. This gender specificity was corroborated by data obtained from human colon cancer patients showing that STAT1 expression is a favorable prognostic marker in males. Interestingly, male but not female Stat1up[[delta]IEC] mice were more resistant to DSS-induced colitis than gender-matched Stat1up[flox/flox] control mice. These mice also displayed reduced infiltration of CD8up[+] T cells into the intraepithelial layer. Taken together, the data demonstrate that the expression of STAT1 in intestinal epithelial cells is vital in modulating the tumor promoting effects of inflammation in a male-specific manner, thus establishing STAT1 as a gender-specific tumor suppressor in colitis-associated colon cancer.

56 STAT1 isoform specific functions in innate and adaptive immunity

Puga, A. (1) Parrini, M. (1), Meissl, K. (1), Lassnig, C. (1,2), Decker, T., (3), Müller, M. (1,2), Strobl, B. (1)

(1) Institute of Animal Breeding and Genetics, University of Veterinary Medicine Vienna, Austria, (2) Biomodels Austria, University of Veterinary Medicine Vienna, Austria; (3) Max F. Perutz Laboratories, University of Vienna, Austria

ana.puga@vetmeduni.ac.at

Signal transducer and activator of transcription (STAT1) is a member of the JAK/STAT signalling cascade and essential for signalling by all types of interferons (IFNs). STAT1 is crucial for the protection against bacterial and viral infections but also contributes to immune-pathologies. STAT1 exists as two alternatively spliced isoforms, STAT1a and STAT1b. They differ in the C-terminal transactivation domain, which is absent in the STAT1b isoform. Accordingly, STAT1b was considered to be transcriptionally inactive if activated in response to IFN-gamma as homodimers and to exert dominant negative functions. Using mice that only express either STAT1a (Stat1a/a) or STAT1b (Stat1b/b) we have shown that STAT1 is transcriptionally active and capable of mediating an IFN-gamma-dependent immune defence against systemic Listeria monocytogenes infections, although with lower efficiency than STAT1a. Herein, we investigate the immune-pathogenic functions of STAT1 isoforms and their role in the regulation of T cell functions. Stat1b/b mice showed an intermediate survival compared to Stat1-/- and Stat1a/a mice upon high-dose lipopolysaccharide challenge, suggesting that STAT1b is less immune-pathogenic than STAT1a. Similar to what we have observed during L. monocytogenes infection, no differences were found between Stat1a/a and wild-type mice, confirming the notion that STAT1b does not act in a dominant negative manner in innate immunity. Notably, we found evidence for a negative regulatory function of STAT1b in splenic CD4+ T cells. Ongoing experiments are directed towards assessing STAT1 isoform specificity in naïve CD4+ T cells, in T helper (Th) cell differentiation and in T cell-mediated diseases in vivo.

57 Systemic metabolic defects caused by epidermal EGFR-deficiency

Jörg Klufa Fabio Savarese, Elena Schmidt, Martin Holcmann and Maria Sibilia

Institute of Cancer Research, Department of Medicine I, Comprehensive Cancer Centre, Medical University of Vienna, Borschkegasse 8a, A-1090 Vienna, Austria

joerg.klufa@meduniwien.ac.at

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. However, these animals develop a severe skin inflammation and a skin barrier defect which become apparent only after mice lacking EGFR in the epidermis 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.

58 Role of the AP-1 protein c-Jun in Imiquimod mediated tumor clearance.

Novoszel, P.

Medical University of Vienna

philipp.novoszel@meduniwien.ac.at

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

Linder, M. Srivatsa, S. (1), Bakiri, L.(2), Dumanic, M.(3), Mitterhauser, M.(3), Wagner, F. E.(2), Sibilia, M.(1)

(1) Institute for Cancer Research, Department of Medicine I, Comprehensive Cancer Center, Medical University of Vienna, Borschkegasse 8a, A-1090 Vienna, Austria

markus.linder@meduniwien.ac.at

60 Visualizing developmental pathway activity in early breast cancer for the characterization of cancer stem cells

Julia Maier (1) Haider Sami (1), Sebastian Gehrig (1), Antonia Geyer (1), Manfred Ogris (1)

(1) Laboratory of MacroMolecular Cancer Therapeutics (MMCT), Department of Pharmaceutical Chemistry, University of Vienna, Vienna, Austria

julia.maier@univie.ac.at

Within a tumor, not all cancer cells are equal. In the last years, the existence of high intra-tumoral heterogeneity in diverse cancer types regarding key parameters has been unraveled. Specifically, a research focus lies in cancer stem cells (CSC), a subset of cancer cells thought to be primarily responsible for crucial pathological features such as latency, cancer mass proliferation, metastases formation, therapy resistance and relapse, revealing CSC as clinically highly interesting. CSC show similarities to healthy stem cells regarding the activity of key developmental pathways, and as such, these pathways offer the possibility not only to understand the still obscure biology of cancer stem cells, but also provide novel targeted therapeutic approaches.In this project, the spatiotemporal pattern of hedgehog, notch and wnt pathway activity in live cells is visualized as cancer develops in 3D. First, a lentivirus based fluorescent reporter construct approach for these pathways is designed and implemented. Focusing at first on a clinically highly relevant cancer type, triple negative breast cancer, cells are transduced and their pathway profile over time is evaluated in vitro in conventional 2D vs. matrigel-based 3D culture. In the next step, in vivo pathway activation in cancer development will be researched, ideally utilizing also patient material, with special focus on early metastases. Lastly, the reporter cell line offers novel screening approaches for innovative substances, which will be explored in the final part of this work.

61 Stromal-derived IGF2 promotes colon cancer progression via paracrine and autocrine mechanisms

Unger, C. (1), Kramer, N. (1), Unterleuthner, D. (1), Sommergruber, W. (2), Schüler, J. (3), Dolznig, H. (1)

(1) Medical University of Vienna, (2) Böhringer Ingelheim RCV GmbH & Co KG, (3) Oncotest GmbH

christine.unger@meduniwien.ac.at

The insulin-like growth factor (IGF)2/IGF1 receptor (IGF1R)-signaling axis plays an important role in intestinal carcinogenesis and overexpression of IGF2 is an accepted risk factor for colorectal cancer (CRC) development. Genetic amplifications and loss of imprinting contribute to the upregulation of IGF2, but insufficiently explain the extent of IGF2 expression in a subset of patients. Here, we show that IGF2 was specifically induced in the tumor stroma of CRC and identified cancer-associated fibroblasts (CAFs) as the major source. Further, we provide functional evidence that stromal IGF2, via the paracrine IGF1R/InsR axis, activated pro-survival AKT-signaling in CRC cell lines. In addition to its effects on malignant cells, autocrine IGF2/IGF1R-signaling in CAFs induced myofibroblast differentiation in terms of Î±-SMA expression and contractility in floating collagen gels. These effects were further augmented in concert with TGFÎ²-signaling suggesting a cooperative mechanism. IGF2-mediated physical matrix remodeling facilitated subsequent tumor cell invasion in organotypic co-cultures. Consistently, mouse xenografts of tumor cells coinoculated with CAFs exhibited increased local tumor regrowth with reduced latency after primary tumor resection when fibroblasts expressed IGF2 as compared to IGF2-silenced CAFs. In line, high-level expression of IGF2 correlated with elevated relapse rates and poor survival in CRC patients. Taken together, we demonstrate that stroma-induced IGF2 promotes colon cancer progression in a paracrine and autocrine fashion and propose IGF2 as potential target for tumor-stroma cotargeting strategies.

62 Pharmacogenetic Analysis of Toxic Encephalopathy in Pediatric Patients Undergoing ALL Treatment

Judit C. SÃ¡gi(1) NÃ³ra Kutszegi(1), Andrea Kelemen(1), BÃ¡lint Egyed(2), AndrÃ¡s Falus(1), GÃ¡bor T. KovÃ¡cs(2), Csaba Szalai(1,3), DÃ¡niel J. Erdélyi(2), Ãgnes F. Semsei(1)

(1)Department of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest, Hungary, (2)2nd Department of Paediatrics, Semmelweis University, Budapest, Hungary, (3)Heim Pal Children Hospital, Budapest, Hungary

sjudit08@gmail.com

The current treatment of pediatric acute lymphoblastic leukemia (ALL) combines several chemotherapic agents often associated with serious side- and adverse-effects. One such adverse event is acute toxic encephalopathy which occurs predominantly during methotrexate-, vincristine-, or cytarabine-administration.Interindividual differences are often observed in the type and grade of the symptoms between patients. In the present study we examined the hypothesis that genetic polymorphism might determine these toxic events via modulation of the pharmacokinetics and pharmacodynamics of drugs used in the treatment protocol.Our study population consisted of 300 pediatric patients with ALL. We evaluated toxic neurological conditions, which were graded according to the Common Terminology Criteria for Adverse Events version 3.0. DNA was isolated from whole blood collected from patients in remission

using QIAmpBlood DNA Maxi Kit. Genotyping was performed using the Sequenom technology. We studied the association between 137 single nucleotide polymorphisms (SNP) and acute toxic encephalopathy. Logistic regression adjusted for potential confounders was performed using the SPSS 20 software.Toxic encephalopathy occured in 9% of the patients. GSTP1 rs1695 G allele (p=8,54E-04; OR=0,15; CI95%=0,05-0,45) and GSTP1 rs749174 A allele (p=1,88E-03; OR=0,2; CI95%=0,07-0,55) SNP appeared to protect against the toxic event. Our results suggest that SNP in GSTP1 impacts the predisposition for developing toxic encephalopathy. Analysis of SNP is recommended for evaluating risks of developing toxic encephalopathy. Currently new data collecting is ongoing to set up a validation cohort with an estimated number of 300 new cases.

63 Increased cholesterol levels are involved in resistance to Destruxins in cancer cells

Heilos, D. (1)(2), Röhrl, C. (3), Pirker, C. (1), Mohr, T. (1), Schwaiger, M. (4), Taibon, J. (5), Windberger, U. (6), Kalvins, K. (7), Baier, D. (6), Eberhart, T. (3), Iqbal, S.M. (2), Stuppner, H. (5), Koellensperger, G. (4), Lemmens-Gruber, R. (2), Dornetshuber-Fleiss, R. (1)(2) and Berger, W. (1)

(1) Institute of Cancer Research, Department of Internal Medicine I, Medical University of Vienna, Comprehensive Cancer Center of the Medical University of Vienna, Austria (2) Department of Pharmacology and Toxicology, University of Vienna, Austria (3) Department of Medical Chemistry, Center for Pathobiochemistry and Genetics, Medical University of Vienna, Austria. (4) Institute of Analytical Chemistry, Faculty of Chemistry, University of Vienna, Währinger Str. 38, 1090 Vienna, Austria. (5) Institute of Pharmacy/Pharmacognosy and Center for Molecular Biosciences Innsbruck (CMBI), University of Innsbruck, Innsbruck, Austria. (6) Decentralized Biomedical Facilities of the Medical University of Vienna, Vienna, Austria. (7) BIOCRATES Life Sciences AG, Eduard-Bodem-Gasse 8, 6020 Innsbruck, Austria

daniela.heilos@univie.ac.at

Recently, ionophoric Destruxins (Dtx) A, B and E, metabolites of entomopathogenic fungi, were proposed for the treatment of malignant tumors. Prior to potential clinical application, resistance mechanisms which may occur during therapy, need to be studied. In the current project we investigated mechanisms of resistance to DtxA, B, or E in a colon carcinoma cell model (HCT116).Sublines were established by exposure selection to increasing concentrations of DtxA, B or E for one year. Cross-resistance profiles to chemotherapeutics, expression of ABC-transporters and gene expression analyses were performed in resistant and parental cells. In addition, basic levels of cholesterol and lanosterol and the de novo synthesis of both metabolites were determined.Lack of cross-resistance to classical anti-cancer drugs was detected, while DtxA- and B-sublines were cross-resistant to DtxB or A, respectively. The DtxE-subline was sensitive to DtxA and B, suggesting similar mechanisms of resistance of DtxA- and B- but not

the DtxE-subline. ABC transporters, frequently involved in chemoresistance, were slightly overexpressed in DtxE- but not A- and B-sublines. Expression arrays suggested hyperactivation of cholesterol synthesis in DtxA- and B-resistant cells (enrichment scores: 0.9 and 0.8). Corroborating, higher levels and synthesis rates of lanosterol and cholesterol were measured in the DtxA-subline. By atomic force microscopy, changes in stiffness and adhesion of cell membranes were shown in all resistant sublines. Accordingly, sensitivity to DtxA and B could be partly restored by Fluvastatin (HMG-CoR inhibitor) and Zometa (a mevalonate pathway inhibitor).Our results indicate that increased cholesterol levels lead to Dtx resistance in cancer cells, likely due to alteration of the cell membrane composition causing reduction of drug uptake or ionophoric activity of Dtx. We next plan to study uptake and ionophoric activity of Destruxins in parental vs. resistant sublines.

64 Mutation of apical pore tyrosine residues turns P-glycoprotein into a nucleotide-gated facilitator

Nasim F(1) Dönmez,Y.(1), Sohail,A.(2), Sitte, HH.(2), Khunweeraphong, N.(4), Kuchler,K.(4), Szakács, G.(3), Stockner, T.(2) and Chiba, P.(1).

(1) Institutes of Medical Chemistry, (2) Pharmacology and (3) Cancer Research, Medical University of Vienna, 1090 Vienna, Austria. (4) Max F. Perutz Laboratories, Medical University of Vienna, Vienna Biocenter, Dr. Bohr-Gasse 9/2, A-1030 Vienna, Austria.

fauzia.nasim@meduniwien.ac.at

ABC transporters translocate substrates at the expense of ATP hydrolysis. The X-ray structure of a homodimeric eukaryotic homolog of human ABCB1 from the red alga Cyanidioschizon merolae suggested a role of apical tyrosine residue Y358 in substrate gating. We mutated analogous tyrosine residues in both the N- and C-terminal halves of human ABCB1 (Y310 and Y953) to alanine and characterized these mutants in rhodamine 123 (rh123) transport experiments. We previously showed that rhodamine 123, as well as other paradigmatic substrates, are able to bind to the transporter in two modes, which are related to each other by 180° rotational symmetry. Introduction of positively charged arginine residues in helix 2 (Q132R) and 8 (Q773R) deselects one of these two binding modes for rh123. The Y310A and Y953A mutations were combined with these mode selector residues. The wild type transporter and mutants were characterized in both a steady state and a zero-trans efflux protocol. All single mutants showed reduced transport activity and higher steady state loading than wild type. One of the mutants (Y953A/Q773R) showed loading which was identical with that of negative controls (cells containing a nonfunctional transporter harboring the E556Q mutation). Nevertheless, the rate for zero-trans efflux was several-fold higher than the rate observed in the absence of a functional transporter. Therefore, this mutant can still transport rhodamine 123 along a concentration gradient, but lacks active transport characteristics. A mutant, in which tyrosine and

selector mutation were combined in inverse manner, showed a similar behavior, while the other two mutants remained active transporters. These experiments for the first time identify apical pore tyrosine residues as gating residues in human ABCB1 and demonstrate that gating occurs in a dual fashion, which is reminiscent of the dual binding mode for substrates and ATP.We acknowledge financial support by the Austrian Science Fund (SFB35).

65 A prosurvival role of SSR2 in melanoma biology

Garg, B (1), Pathria , G.(2), Wagner, C.(3), Maurer, M.(4), Wagner, S.N.(5)

DIAID, Department of Dermatology, Medical university of Vienna

n1242781@students.meduniwien.ac.at

Signal Sequence Receptor 2 (SSR2) was revealed as a possible driver of melanoma metastasis in a subset of patients as exhibited by the systematic search algorithm, INtegrated DEtection of Genomic Outliers (INDEGO). INDEGO is a sequential search across human tumor samples for transcript outlier data points with associated gene copy number variations that are correlated with patient's survival to identify genes with pro-invasive functionality. Encouraged by a successful proof of concept study with validation of MTSS1 as driver of metastasis in human melanoma and the high confidence shown by a Cox Proportional Hazards Model Analysis displaying a statistically significant negative association of SSR2 transcript levels with survival of primary melanoma patients ($p = 0.0098$, $HR = 0.115$, 95% $CI = 0.022 - 0.593$), we hypothesized that SSR2 upregulation could be a driver mechanism in human melanoma. Pro-survival effects of SSR2 were examined through FACS-based analysis for induction of apoptosis. SSR2 knockdown led to increased cell death in human melanoma cells and, consistently, increased expression of SSR2 was associated with drug resistance. Given the established role of SSR2 in protein gating to ER, as a part of the SSR complex, we hypothesized protection against ER stress as a possible mode of action of SSR2. Furthermore, we found a statistically significant gene expression correlation between SSR2 and the transcription factor XBP1 in primary melanoma samples with SSR2 outlier expression. X-Box Binding Protein 1(XBP1) is induced by stress and the key effector molecule of the IRE1α branch of the Unfolded Protein Response (UPR). Notably, we also observed that induction of stress in human melanoma cells led to XBP1 upregulation followed by an increase in SSR2 transcript and protein levels. Together with these data and the fact that transcriptional activity of XBP1s has been shown to have pro-tumorigenic effect, we propose SSR2 as a possible target for melanoma treatment.

66 Chromatin accessibility maps of chronic lymphocytic leukemia identify subtype-specific epigenome signatures and associated transcription regulatory networks

Rendeiro A., Schmidl C., Strefford J. C., Walewska R., Davis Z., Farlik M., Oscier D., Bock C.

CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria, Cancer Sciences, Faculty of Medicine, University of Southampton, Southampton, United Kingdom, Department of Molecular Pathology, Royal Bournemouth Hospital, Bournemouth, United Kingdom, Department of Laboratory Medicine, Medical University of Vienna, Vienna, Austria, Max Planck Institute for Informatics, Saarbrücken, Germany

arendeiro@cemm.oeaw.ac.at

Chronic lymphocytic leukemia (CLL) is characterized by substantial clinical heterogeneity, despite relatively few genetic alterations. To provide a basis for studying epigenome deregulation in CLL, we established genome-wide chromatin accessibility maps for 88 CLL samples from 55 patients using the ATAC-seq assay, and we also performed ChIPmentation and RNA-seq profiling for ten representative samples. Based on the resulting dataset, we devised and applied a bioinformatic method that links chromatin profiles to clinical annotations. Our analysis identified sample-specific variation on top of a shared core of CLL regulatory regions. IGHV mutation status – which distinguishes the two major subtypes of CLL – was accurately predicted by the chromatin profiles, and gene regulatory networks inferred for IGHV-mutated vs. IGHV-unmutated samples identified characteristic differences between these two disease subtypes. In summary, we found widespread heterogeneity in the CLL chromatin landscape, established a community resource for studying epigenome deregulation in leukemia, and demonstrated the feasibility of chromatin accessibility mapping in cancer cohorts and clinical research.

67 Functional Analysis of the Conserved Interactome of NUP98-Fusion Proteins in AML

Terlecki-Zaniewicz (1), S. (1) Schmöllerl, J. (1), Grebien, F. (1)

(1) Ludwig Boltzmann Institute for Cancer Research, Vienna

stefan.terlecki-zaniewicz@lbicr.lbg.ac.at

Chromosomal rearrangements in cancer can lead to the fusion of two gene loci, resulting in the production of a pathogenic fusion protein. In leukemia, a particular high number of fusion oncogenes have been identified. Fusion proteins involving the NUP98 gene (Nucleoporin 98) are found in ~2% of acute myeloid leukemia (AML) patients. The NUP98 multi-partner translocation family (MPTF) features >20 different fusion proteins, all harbouring the N-terminal

part of NUP98 fused to distinct C-terminal fusion partners. Previous studies indicated that different NUP98-fusions cause a similar AML phenotype in human and mouse models. Thus, we postulate that all NUP98 fusion proteins share molecular mechanisms to modulate important oncogenic pathways. The aim of our work is to identify critical common effectors of the NUP98 MPTF among the interactome of five distinct NUP98-fusion proteins using affinity purification-coupled to mass spectrometry (AP-MS). Doxycycline (Dox)-inducible, Strep-HA-tagged variants of five selected NUP98 fusion proteins (NUP98-HOXA9, NUP98-JARID1A, NUP98-DDX10, NUP98-NSD1 and NUP98-PSIP1) were cloned into retroviral vectors. Human leukemia cells were transduced with the constructs and selected for transgene integration. As the expression of fusion proteins is coupled to GFP expression, Dox-mediated transgene induction was reported by GFP expression. This analysis revealed that all cell lines, established so far, induced high levels of GFP expression of 80%-90% 24 hrs after Dox treatment. We are currently using these cell line models to optimize biochemical procedures in order to achieve efficient purification of NUP98-fusion protein complexes.In the future we aim to functionally dissect the NUP98 interactome by performing a loss-of-function screen to identify targets for in-depth in vitro and in vivo validation studies.

68 Identification of a novel STAT5 inhibitor to interfere with the oncogenic activities of STAT5 in AML

Bettina Wingelhofer (1,2), Elizabeth C. Heyes (1,2), Barbara Maurer (1,2), Andrew M. Lewis (3), Abbarna A. Cumaraswamy (3), Sadovnik Irina (4), Stefan Kubicek (5), Peter Valent (4), Patrick T. Gunning (3), Richard Moriggl (1,2,6)

(1) Ludwig Boltzmann Institute for Cancer Research, 1090 Vienna, Austria, (2) Institute of Animal Breeding and Genetics, University of Veterinary Medicine Vienna, 1210 Vienna, Austria, (3) Department of Chemistry, University of Toronto Mississauga, Mississauga, Ontario L5L 1C6, Canada, (4) Department of Internal Medicine I, Division of Hematology & Hemostaseology, Medical University of Vienna, Austria, (5) CeMM Research Center for Molecular Medicine of the Austrian Academy of Scienes, 1090 Vienna, Austria, (6) Medical University of Vienna, 1090 Vienna, Austria

bettina.wingelhofer@lbicr.lbg.ac.at

STAT5 is frequently hyper-activated in a variety of hematopoietic cancers as a result of deregulated tyrosine kinase signaling. To date, various tyrosine kinase inhibitors (TKIs) are in the clinic or in clinical trials for treatment of hematopoietic diseases but TKI treatment is often accompanied by resistance development and cytotoxicity. Bypassing tyrosine kinases through

direct inhibition of STAT5 would be advantageous for therapy development especially in the case of STAT5 regulated cancers. In collaboration with Prof. Patrick Gunning, a library of lead STAT5 inhibitors targeting the SH2 domain has been established, which was extensively validated in vitro on AML model cell lines and patient samples. Therefore, standard techniques, such as Western Blotting, IP, EMSA, qRT-PCR, as well as AnnexinV/PI and PI staining were used. Furthermore, combinatorial effects of the selected STAT5 inhibitor with a library of >1800 experimental or FDA approved drugs were evaluated. We identified a small inhibitory molecule, called AC-4-130, which binds to the SH2 domain of STAT5, causing the disruption of the reciprocal STAT5-phosphopeptide interactions. AC-4-130 efficiently blocked phosphorylation, dimer formation, nuclear translocation, DNA binding and target gene expression. Furthermore, AC-4-130 led to a cell cycle blockade in G0/G1 and the induction of apoptosis. Studies with human AML patient-derived samples similarly showed the induction of apoptotic cell death and decreased colony forming capabilities. A combinatorial drug screen revealed synergistic effects of AC-4-130 with TKIs, as well as with drugs standardly used in the clinical treatment of AML patients. In summary, our findings indicate that AC-4-130 is a potent and selective inhibitor of STAT5. This compound provides a lead structure for further chemical modifications and clinical development to improve existing therapies and overcome resistance development in hematopoietic malignancies.

69 C/EBP[alpha] N-terminal leukemia is sensitive to small molecule-inhibition of the MLL-Menin interaction

Schmidt, L. (1) Grebien, F. (1)

(1) Ludwig Boltzmann Institute for Cancer Research, Vienna, Austria.

luisa.schmidt@lbicr.lbg.ac.at

The gene encoding for the transcription factor C/EBP[alpha] is mutated in 9% of patients with acute myeloid leukemia (AML). Use of different ATG codons leads to expression of two C/EBP[alpha] isoforms: a full-length version, termed p42, and a shorter form, termed p30. A balanced ratio of C/EBP[alpha] isoforms is crucial for hematopoietic homeostasis. In AML patients, CEBPA N-terminal mutations lead to selective loss of p42 expression without affecting p30 translation. p30 was recently shown to preferably interact with WDR5, a component of the MLL/SET histone methyltransferase complex. Disruption of this interaction induced myeloid differentiation in murine and human C/EBP[alpha]-mutant AML cells. Thus, our hypothesis is that C/EBP[alpha]-mutant AML is particularly sensitive to perturbation of MLL/SET function. MI-463 and MI-503 are two potent and orally bioavailable small-molecule inhibitors of the menin-MLL interaction. In our study, we will investigate the effect of MLL/SET perturbation by MI-463 and MI-503 on N-terminal CEBPA mutated AML in Cebp[alpha]p30/p30 cells.We show that cells from a Cebp[alpha]p30/p30 AML mouse model are 2-6 fold more sensitive towards the

MI-463 and MI-503 than other leukemia cell lines of mouse and human origin. Both inhibitors lead to a time- and dose-dependent impairment of proliferation, increased apoptosis as well as induction of myeloid differentiation as measured by increased surface levels of Mac-1 and Gr-1. We are currently investigating the impact on overall gene expression by RNA-seq and the effect on human CEBPA mutated AML cells.Overall, we could show that N-terminal CEBPA mutated AML is particularly sensitive to perturbation of the MLL/SET complex by inhibition of the MLL-Menin interaction. These findings contribute to a better understanding of N-terminal CEBPA mutated AML and may inform new therapeutic strategies for leukemia treatment.

70 Identification of invasion-addicted genes driven by transforming growth factor-beta in hepatocellular carcinoma

Christine Haider (1), Cédric Coulouarn (2), Markus Grubinger (1), Karin Schlangen (3) and Wolfgang Mikulits (1)

(1) Department of Medicine I, Division: Institute of Cancer Research, Comprehensive Cancer Center Vienna, Medical University of Vienna, Borschkegasse 8a, 1090 Vienna, Austria, (2) Inserm, UMR991, Liver Metabolisms and Cancer, Rennes, France, (3) Section of Biosimulation and Bioinformatics, Center for Medical Statistics, Informatics and Intelligent Systems (CeMSIIS), Medical University of Vienna, Spitalgasse 23, A-1090 Vienna, Austria

christine.haider@meduniwien.ac.at

Transforming growth factor (TGF)-[beta] is a ubiquitously expressed cytokine with fundamental roles in cell physiology. In hepatocellular carcinoma (HCC), TGF-[beta] signaling plays a dual role. It suppresses carcinogenesis at early stages by inducing growth arrest and apoptosis, but triggers the gain of metastatic abilities at later stages. The molecular mechanisms underlying this 'TGF-[beta]-switch' are only beginning to be unravelled. To mimic the pathophysiological situation as closely as possible, we exposed HCC cells to TGF-[beta] for long-time. Through this approach we aimed at identifying cooperating factors and signaling pathways that cause the switch to TGF-[beta]-dependent cell invasion. Therefore we established two cellular models of human HCC invasion, where cells were exposed to long-term TGF-[beta] treatment. Major features of both HCC models include a high degree of dedifferentiation, an autocrine TGF-[beta] loop and the dependency of their migratory to TGF-[beta]. Importantly, the two models show opposite effects after long-term exposure to TGF-[beta], as one shows enhanced migration while the other shows a reduced one. Furthermore, both HCC cell types still show reduced proliferation proposing that cells can evade specific paths of the TGF-[beta]-induced tumor suppression. To identify genes responsible for the tumor-promoting mechanisms of TGF-[beta], we determined the changes in gene expression of HCC cells with and without long-term TGF-[beta] treatment.

To filter those genes that are relevant in HCC patients, we correlated the expression data with information on HCC patient survival from the TCGA platform. We identified six “invasion-addicted” genes including CXCL5 and SLC22A15 which will be further investigated. In conclusion, long-term exposure of HCC cells to TGF-[beta] shows a partial evasion from anti-oncogenic traits together with the gain of pro-oncogenic, invasive abilities, demonstrating the complexity of TGF-[beta] functions.

71 Development of an affinity purified Antibody for the detection of Metastatic Melanoma

Schütz, B. (1) Koppensteiner, A. (2), Missbichler, A. (3), Hundsberger, H. (2), Mikula, M. (1)

(1) Institute of Medical Genetics, Medical University of Vienna, Vienna, Austria (2) Medical and Pharmaceutical Biotechnology, University of Applied Sciences, Krems, Austria, (3) former address: Sciotec Diagnostic Technologies GmbH, Tulln, Austria

birgit.schuetz@meduniwien.ac.at

Melanoma is the most aggressive type of skin cancer and also one of the most frequent tumors in young adults. Even though it only accounts for 4% of all cases of skin cancer, melanoma is responsible for the majority of all skin cancer related deaths. Identification of primary tumors prone to develop metastasis is of paramount importance for patient stratification. However, there are no molecular markers routinely used to predict clinical progression of the disease. We generated antiserum directed against metastatic melanoma tissue lysate. Furthermore, we set up a novel approach to purify the obtained serum via affinity chromatography in order to generate a malignant melanoma specific antibody, termed MHA-3. The established antibody shows high sensitivity and specificity in immuno-stainings when tested on biopsies of melanoma patients. We compared staining of MHA-3 to αS100b, an antibody commonly used to detect melanoma, and observed that it reproducibly recognizes melanoma patients which developed metastasis. Analysis of antigen bound by MHA-3 revealed 18 distinct proteins. Importantly, the combined expression profile of all identified antigens is superior compared to individual antigens when subjected to Kaplan Meier analysis. In summary, we developed a polyclonal antibody which is able to detect metastatic melanoma on paraffin embedded sections. Hence, we propose that this antibody will represent a valuable tool in melanoma diagnosis.

72 A quest to elucidate the role of the non-canonical nucleotide binding site of the bile salt export pump (BSEP/ABCB11) in ATPase activity and taurocholate transport

Sohail, MI. (1), Wlcek, K (2), Telbisz, A. (3), Sarkadi, B. (3), Ecker, G. (2), Stockner, T. (4), Chiba, P. (1)

(1) Institute of Medical Chemistry, Medical University of Vienna, Waehringerstrasse 10, Vienna, Austria, (2) Department of Pharmaceutical Chemistry, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria (3) Institute of Enzymology, RCNS-Hungarian Academy of Sciences, Magyar Tudosok krt. 2, H-1117, Budapest, Hungary (4) Institute of Pharmacology, Center for Physiology and Pharmacology, Medical University of Vienna, Waehringerstrasse 13A, 1090 Vienna, Austria

muhammad.sohail@meduniwien.ac.at

ATP-binding cassette (ABC) transporters bind and hydrolyze ATP to energize transmembrane transport of cargo. ATP binds at two rotationally symmetric nucleotide binding sites (NBSs) at the interface of the nucleotide binding domains. About half of the human ABC proteins harbor a non-canonical NBS1. We investigate the role of this NBS in BSEP, the human bile salt export pump (BSEP, ABCB11) for bile salt transport. The NBD interfaces of ABCB1 and BSEP differ in only 4 residues, all of which are located in NBS1 (E502 (Q-loop), M584 (Walker B) and R1221 and E1223 (C-motif)). These residues are S, E, G and Q, respectively in the canonical site of ABCB1. We generated the M584E single and the E502S.M584E.R1221G.E1223Q quadruple mutation. Wild-type and BSEP mutants were expressed in HEK293 cells and membrane vesicles were prepared (Hirano et al., 2005). The surface expression was determined by Western blotting and [3H]taurocholate uptake activity was measured using a rapid filtration technique (Gerloff et al., 1998). Substitution of non-canonical residues of NBS1 with canonical ones decreased both TC transport and ATPase activity. While the single mutant showed 90% of wt transport activity, transport in the quadruple mutant decreased to 35 percent. We also exchanged the catalytic glutamate between NBS2 and NBS1. Unexpectedly, the ensuing double mutant M584E/E1244Q showed about 15% residual transport activity as compared to wild-type protein. We conclude that during evolution the non-canonical nucleotide binding site was optimized structurally and functionally to provide a platform for ATP hydrolysis in the consensus site. In addition, our experiments for the first time show that NBS1 can functionally replace NBS2 in mediating taurocholate transport. Implications of these findings for the transport mechanism will be discussed. Supported by grants from the Austrian Science Fund (FWF) within the scope of SFB35. MIS is supported by the Higher Education Commission Pakistan.

73 JAK2 in non-small cell lung cancer, friend or foe?

Julian Mohrherr Emilio Casanova

Medical University Vienna

julian.mohrherr@lbicr.lbg.ac.at

Lung cancer represents the leading cause for cancer related deaths worldwide. Non-small cell lung cancer (NSCLC), which is the most common NSCLC, is associated with a very bad prognosis. A better understanding of molecular mechanisms responsible for the initiation and progression of NSCLC is critical in order to identify novel drug targets. Somatic Janus kinase 2 (JAK2) mutations are found in approximately 7% of the NSCLC patients, however their relevance to tumor development and progression is poorly addressed. Nevertheless, the JAK2 inhibitors Ruxolitinib (Jakavi®) and Tofacitinib (Xeljanz®) are currently in clinical trial phase I/II for the treatment of NSCLC. Accordingly, we aim to investigate the role of JAK2 in Kirsten rat sarcoma viral oncogene homolog (KRAS) driven lung tumorigenesis. Patient data from smokers which are known to be more prone to harbor KRAS mutations indicate that low JAK2 expression levels significantly correlate with reduced overall survival. However, a distinction between stromal or tumor cell intrinsic effects of JAK2 cannot be made in this setting. Interestingly, pharmacological inhibition of JAK2 with Ruxolitinib (Jakavi®) and Tofacitinib (Xeljanz®) in a panel of human and mouse KRAS-mutated NSCLC cell lines (A549, A427, SK-LU-1, 368T1) attenuates tumor cell proliferation and triggers enhanced apoptosis. These data might indicate divergent functions of stromal and tumor cell intrinsic JAK2 signaling. To follow up on these findings we are currently investigating in vivo the impact of JAK2 deletion, specifically in KRAS mutated tumor cells taking advantage of KrasLSL-G12D/+ mice crossed with JAK2fl/f mice. Additionally, an inducible systemic knockout of JAK2 is performed by crossing KrasLSL-G12D/+: JAK2fl/+ mice with ROSA-CreERT2 mice.Altogether, we aim to clarify the benefit of JAK2 inhibitor treatment and eliminate potential hazards, specifically in NSCLC patients harbouring KRAS mutations.

74 Rho regulated transcription via MRTFB is suppressed by EWS-FLI1 in Ewing Sarcoma

Katschnig, A.M. (1), Kauer, M.O. (1), Schwentner, R. (1), Tomazou, E.M. (1), Lawlor, E.R. (3), Aryee, D.N.T. (1,2), and Kovar, H. (1,2)

(1) Children's Cancer Research Institute, St. Anna Kinderkrebsforschung, Vienna, Austria, (2) Department of Pediatrics, Medical University Vienna, Vienna, Austria, (3) Department of Pediatrics, University of Michigan, Ann Arbor, MI, USA

anna.katschnig@ccri.at

Ewing Sarcoma (ES) is an aggressive pediatric bone tumor. The driver of ES oncogenesis is the chimeric gene product EWS-FLI1 encoding an aberrant ETS transcription factor. Previous studies suggest that EWS-FLI1 alters ES cell morphology by affecting the actin cytoskeleton. The Rho pathway is the major regulator of the actin cytoskeleton and several Rho/SRF target genes are repressed in the presence of EWS-FLI1. Serum-induced Rho activation leads to polymerization of actin monomers, which triggers nuclear translocation and SRF binding of the

myocardin-related transcription factors A and B (MRTFA/B). In order to study a potential deregulation of the Rho/actin/MRTFA/B axis by EWS-FLI1 on a transcriptional level, we studied genome-wide gene expression in A673 and SKNMC ES cell lines in presence and absence of EWS-FLI1 and MRTFA/B under serum on and off conditions. We found that transcriptional MRTF activity was largely serum independent and low in the presence of EWS-FLI1. Strikingly, gene expression changes caused by EWS-FLI1 silencing were partly rescued in absence of MRTFB suggesting that MRTFB function is overall repressed by EWS-FLI1. By chromatin immunoprecipitation with DNA sequencing (ChIP-seq) of MRTFA/B, SRF and EWS-FLI1 we found that the overlap of MRTFB ChIP-seq peaks with SRF was much lower than with EWS-FLI1. Upon serum stimulation, MRTFB binding was significantly enriched in TEAD motifs, especially in the absence of EWS-FLI1. Among the inversely regulated MRTFB/EWS-FLI1 target genes were several bona-fide targets (CYR61, CTGF, ANKRD1) of the YAP/TAZ/TEAD pathway. Hence we hypothesize that MRTFB is inhibited by EWS-FLI1 and upon EWS-FLI1 knockdown potentially assembles with other transcription factors ultimately resulting in the observed global effects on EWS-FLI1 dependent gene expression. Given our preliminary results TEAD transcription factors are likely candidates for the interaction with MRTFB.

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

Redl, E. (1) Dillinger, T.(1), Hassler, M.R.(1), Kenner, L.(1, 2, 3), Egger, G.(1)

(1) Clinical Institute of Pathology, Medical University of Vienna. Ludwig Boltzmann Institute for Cancer Research, Vienna. Department of Laboratory Animal Pathology, University of Veterinary Medicine, Vienna.

elisa.redl@meduniwien.ac.at

NPM-ALK positive anaplastic large cell lymphoma is a rare and aggressive Non-Hodgkin's lymphoma of T cell origin, which is driven by constitutive activation of the oncogenic anaplastic lymphoma kinase ALK through deregulation of several pathways promoting cell proliferation and survival.Recent work suggested that ALK signaling can directly impact on epigenetic alterations, such as DNA methylation in tumor cells. There is evidence that STAT3, the key downstream mediator of ALK signaling, regulates and targets the DNA methyltransferase DNMT1 to promoters of tumor suppressor genes inducing their silencing. Furthermore, in cell lines chemical inhibition of DNMT1 by 5-Aza-2'-Deoxycytidine impairs ALK signaling and causes loss of STAT3 activity, resulting in cell cycle arrest and apoptosis.We show that T cell specific deletion of the maintenance methyltransferase gene Dnmt1 abolishes tumor formation in a transgenic NPM-ALK lymphoma mouse model (NPM-ALK;Dnmt1[up-/-]). Intriguingly, we did not observe altered proliferation or apoptosis in Dnmt1 deficient NPM-ALK thymocytes.

Further, the oncogene NPM-ALK and its downstream target STAT3 were highly active in the targeted T cells, indicating that downstream ALK signaling via STAT3 is maintained upon Dnmt1 deletion in NPM-ALK expressing T cells in vivo. We are currently performing gene expression profiling using RNA sequencing as well as reduced representation bisulfite sequencing (RRBS) to identify genome wide changes in gene expression and aberrant DNA methylation patterns in NPM-ALK and NPM-ALK;Dnmt1[up-/-] mice. Integration of DNA methylation (RRBS) and gene expression (RNA-Seq) will allow us to infer the relation of oncogenic signaling and epigenomic aberrations. Together, our data suggest that aberrant DNA methylation is critically involved in ALK dependent lymphomagenesis and DNMT1 might be essential for the fusion kinase to exert its oncogenic potential.

76 The role of DNMT1 for survival of anaplastic large cell lymphoma cells

Dillinger, T. (1),Redl, E.(1), Hassler, MR.(1), Tran, L.(1), Kenner, L.(1,2,3), Egger, G.(1)

Clinical Institute of Pathology - Medical University of Vienna, Ludwig Boltzmann Institute for Cancer Research Vienna, Department of Laboratory Animal Pathology - University of Veterinary Medicine Vienna

thomas.dillinger@meduniwien.ac.at

DNA methylation is an epigenetic process, by which methyl groups are added to the C5 position of cytosines on the DNA. Methylation modifies the function of the DNA by altering gene expression and is essential for normal gene regulation and development. Hypomethylation is usually associated with transcriptionally active DNA, while hypermethylation causes gene repression. Aberrant DNA methylation patterns are widely observed in a variety of tumors. This includes hypermethylations of CpG-Islands in promoters of tumor suppressor genes leading to epigenetic silencing.NPM-ALK positive T-cell lymphoma is a form of Non-Hodgkin's lymphoma and subgroup of the anaplastic large cell lymphoma (ALCL). Frequently ALCL patients possess a genetic translocation between the anaplastic lymphoma kinase (ALK) and a nuclear transport protein, Nucleophosmin1 (NPM). This fusion protein leads to activation of several pathways such as STAT3 signaling, resulting in proliferation and survival. Using a transgenic NPM-ALK mouse model we showed that the deletion of the maintenance methyltransferase Dnmt1 inhibits lymphomagenesis and thereby sustained survival of the mice.To investigate how depletion of DNMT1 prohibits NPM-ALK tumorigenesis, we analyzed tumor cells from NPM-ALK mice by inhibiting DNMT1 chemically with 5-aza-2'-deoxycytidine and on a genetic level making use of an inducible Cre recombinase. Depletion of DNMT1 resulted in increased cellular death and loss of overall DNA-methylation levels. Interestingly, 5-aza-CdR treatment resulted in a reduction of phospho-ALK and phospho-STAT3 while genetic Dnmt1 deletion couldn't show similar effects. RNA-sequencing and RRBS data are currently being analyzed and might give possible explanations for the observed differences.Together our

data suggest that DNMT1 and its resulting DNA methylation pattern are essential for both lymphoma development and tumor cell survival in ALCL.

77 Impact of Gallic acid, a constituent component of plants food and beverages on obesity induced DNA damage

Setayesh, T (1), Ferk, F.(1), Nersesyan, A. (1), Haslberger, A. (2), Huber, H. (1), Haslinger, E. (1), Knasmüller, S.(1)

(1) Institute of Cancer Research, Department of Medicine I, Medical University Vienna, Borschkegasse 8a, 1090 Vienna, Austria, (2) Department of Nutritional Sciences, University of Vienna, Althanstraße 14,Vienna 1090, Austria

n1249216@students.meduniwien.ac.at

78 Novel cell death mechanism of fatty acid synthase inhibition in cancer cells

Kührer, K., Wilfinger, N., Billerhart, M., Nowikovsky, K.,

Department of Internal Medicine I, Medical University Vienna, Austria

kristina.kuehrer@gmx.at

79 STAT5B N642H is a driver mutation for leukemia

Pham, H. T. T. (1,2), Barbara Maurer (1,2), Michaela Prchal-Murphy (3), Eva Grundschober (3), Harini Nivarthi (4), Tahereh Javaheri (1,2), Jan Pencik (1), Zahra Kazemi (2,5), Florian Grebien (1), Lukas Kenner (1,2,7), Peter Valent (2,8), Auke Boersma (6), Thomas Kolbe (6), Mathias Müller (6), Thomas Rülicke (6), Veronika Sexl (3) and Richard Moriggl (1,2,6)

(1)Ludwig Boltzmann Institute for Cancer Research, Vienna, Austria. (2)Medical University Vienna, Vienna, Austria. (3)Institute of Pharmacology and Toxicology, University of Veterinary Medicine, Vienna, Vienna, Austria. (4)CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences Vienna, Austria. (5)Center of Physiology and Pharmacology, Vienna, Austria. (6)Institute of Animal Breeding and Genetics & Biomodels Austria, University of Veterinary Medicine Vienna, Vienna, Austria. (7)Clinical Institute of Pathology, Vienna, Austria and Unit of Pathology of Laboratory Animals, University of Veterinary Medicine, Vienna, Austria . (8)Department of Medicine I, Division of Hematology and Ludwig-Boltzmann Cluster Oncology, Medical University of Vienna, Austria

ha.pham@lbicr.lbg.ac.at

80 Targeting the vulnerable transcriptional core of the NUP98 Translocation Family in Acute Myeloid Leukemia

Schmoellerl, J. (1) Barbosa, I. (2), Zuber, J. (2), Grebien, F. (1)

(1) Ludwig Boltzmann Institute for Cancer Research (LBI-CR), Vienna, Austria, (2) Research Institute of Molecular Pathology (IMP), Vienna, Austria

johannes.schmoellerl@lbicr.lbg.ac.at

81 Virus on a lipid diet: the role of N-myristoylation in Picornaviridae infectious cycle

Irena Čorbić Ramljak (1), Julia Stanger (1), Dominik Dreier (2), Heinrich Kowalski (1)

(1) Department of Medical Biochemistry, Max F. Perutz Laboratories, Medical University of Vienna, Austria.; (2) Institute of Applied Synthetic Chemistry, Technical University of Vienna, Vienna, Austria.

irena.corbic@meduniwien.ac.at

82 Exploration of features of HUVEC senescence and their evaluation as markers by computer-assisted single-cell assessment

Oszwald, A. (1), Awad, E.M. (2), Khan, S.Y. (3), Breuss, J.M. (4)

Medical University of Vienna, Center for Physiology and Pharmacology, Department of Vascular Biology

n0942245@students.meduniwien.ac.at

The increasing effort dedicated to senescence research has not been met with equal efforts to further define the phenomenon, or more closely characterize frequently employed markers. The purpose of this study is to provide a model of Human Umbilical Vein Endothelial Cell (HUVEC) senescence features, and their modulation by culture density or TNF-Î±, using computer assisted image analysis.HUVEC were subjected to repeated subculture until replicative exhaustion was observed. Cells of passage 5 and 20 processed for ICC or cytochemical Î²-galactosidase.Assessment of p16 and p21 suggests that p16 is a robust feature of late-stage senescence, while p21 levels, albeit consistently higher in P20, are more variable in young cells.

A model is proposed for classification for distinct Î³H2AX patterns, which relate to different states of p21 and Ki67 levels, or appear as passage dependent features. Experimental TNF-Î± stimulation suggested that while senescent HUVEC exhibit higher baseline levels of nuclear NFÎºB (p65, p52) and higher E-Selectin and ICAM-1 levels per cell, they were not modulated in response to stimulation. Alteration of culture density suggested that certain features implicated in HUVEC senescence may be variably sensitive to density over a range of passages (P5, P8 and P20). Specifically, high cell densities resulted in increased cytochemical Î²-galactosidase stain (which coincided with increased CD31 levels in P5 and P8), reduced levels of PAI-1, and to a lesser extent, p21.In this study, multiple features of senescent HUVEC were assessed on the single cell level in two very distinct cultures. This allows sampling a broad spectrum of senescence-related phenotypes, and demonstrates the importance of combining markers when evidencing senescence. Characterization of senescent HUVEC phenotypes based on (stimulus dependent) features may allow delimiting early and late states in senescence progression, and yield insight into underlying mechanisms.

83 ATGL deficiency leads to lung cancer in mice

Bina, I. (1), Al-Zoughbi, W.(1), Schauer, S.(1), Stadelmeyer, E.(1), Nicholson Puthen Veedu, S.(1), Kashofer, K.(1), Marsh, L.(3), Schlederer, M.(4), Birner-Gruenberger, R.(1), Schweiger, M.(2), Kenner, L.(4), Zechner, R.(4), Hoefler, G.(1) and Vesely, P.W.(1,2)

(1)Medical University of Graz, Institute of Pathology, Auenbruggerpatz 25, 8036 A-Graz, (2)University of Graz, Institute of Molecular Biosciences, HeinrichstraÃŸe 31/II, 8010 A-Graz, (3)Ludwig Boltzmann Institute Lung Vascular Research, Stiftingtalstrasse 24 8010 A-Graz, (4)Ludwig Boltzmann Institute Cancer Research, Waehringerstr. 13a, 1090 A-Vienna

isabelle.bina@medunigraz.at

84 The effect of high-dose remifentanil on the reversal of neuropathic pain in post herpetic patients

Prosenz, J. (1), Kloimstein, H. (2), Thaler, U. (3), Drdla-Schutting, R. (4), Sandkühler, J. (5), Gustorff, B. (6)

(1) Medical University of Vienna (2,3,6) Wilhelminen hospital, (4,5) Medical University of Vienna

julian.prosenz@meduniwien.ac.at

Aim: Opioids are the gold standard for symptomatic therapy of moderate to severe pain. Now, a new effect has been discovered in animals – the reversal of long-term potentiation (LTP) at C-fiber synapses. This opens the possibility for novel therapeutic strategies. The aim of this study was to explore the effects of remifentanil in chronic post-herpetic pain patients. Methods: In this uncontrolled, open-label, exploratory pilot study patients with spontaneous pain >30 (NRS, 0-100) were treated with a one-hour high-dose remifentanil infusion and followed up for one week. Mechanical hyperalgesia (=mechanical pain sensitivity, MPS) was measured with 7 modified rigid von Frey filaments (8-512 mN), sensory patterns were assessed with Quantitative Sensory Testing (QST) (warm, cold, heat pain, cold pain perception, and heat and cold pain tolerance thresholds, mechanical pain threshold, and dynamic mechanical allodynia). Response was defined as a [>=]30% reduction in pain intensity on day seven after treatment.Results: Of 20 treated patients, 11 responded to treatment (55%). The mean overall change in pain intensity was -17.5 (-7.1 – -27.9, 95I;) one day and -18 (-7.5 – -28.5, 95I;) seven days after treatment ($F(2,19) = 13.17$, $p = 0.0001$), with the responders experiencing a mean pain reduction of 61% at day seven. In the responder group there was weak evidence of a normalization of MPS after treatment, with less patients showing abnormal MPS ($p = 0.08$). Surprisingly, non-responders were more hyperalgesic (MPS) at baseline than responders ($p < 0.05$). We did not identify any other predictors of treatment response. Conclusion: In this pilot study we were able to successfully translate basic science knowledge. The high number of clinically meaningful, good, and excellent responders suggests that a novel, curative treatment approach for chronic pain may be at hand. We believe the current level of evidence supports the conduction of a randomized controlled trial.

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

Hafiz Muhammad Mazhar Asjad Ali El-Kasaby, Ameya Kasture, Oliver Kudlacek, Sonja Sucic, Michael Freissmuth

Institute of Pharmacology, Medical University of Vienna

n1442638@students.meduniwien.ac.at

Uptake through the dopamine transporter (DAT) represents the primary mechanism used to terminate dopaminergic transmission in the brain. DAT has a PDZ-binding motif on its C-terminus, which is required for cell surface delivery. Substitutions within the PDZ-domain or extensions at the C-terminus result in intracellular retention. In contrast, the serotonin transporter(SERT) is the only monoamine transporter, which tolerates modifications of its C-terminus. Our working hypothesis posits that DAT and its close relative NET (norepiphrine transporter) recruits the exocyst complex via its C-terminus. Accordingly, we examined the effects of exocyst components on transporter expression by performing radiolabelled substrate

uptake assays in HEK293 cells (a cell line of fibroblast origin) or in CAD cells (a CNS cathecholaminergic cell line, which endogenously expresses the NET and many neuron-specific proteins, such as class III beta-tubulin, GAP-43, SNAP-25, and synaptotagmin). Briefly, the cells were transiently transfected with the plasmids encoding DAT, SERT or NET, along with different amounts of the plasmid encoding Exo70; 48h after transfection, uptake of radiolabelled substrate was determined to quantify surface expression of the transporters. The data indicated that DAT relied on the exocyst to reach the cell surface. Surprisingly, SERT did not require the exocyst complex to reach the cell surface, regardless of whether the experiments were performed in HEK293 cells or in CAD cells. Moreover, confocal laser scanning microscopy in HEK293 cells transiently transfected with DAT or SERT, in the absence or presence of different amounts of dominant negative Exo70, also confirmed that DAT, but not SERT, was dependent on Exo70. These experiments are of physiological relevance because misfolded DAT mutants have been shown to cause Parkinson´s disease. Hence, we should expect these folding-deficient versions of DAT to fail binding the exocyst complex, in turn leading to their

86 Unraveling the Role of Epigenetic Modifiers in BAF-mutant Cancers

Boidol, B. (1), Dönertas, D. (2), Kubicek, S. (3)

(1) CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria - Christian Doppler Laboratory for Chemical Epigenetics and Antiinfectives, (2) CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria - Christian Doppler Laboratory for Chemical Epigenetics and Antiinfectives

bboidol@cemm.oeaw.ac.at

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 signaling 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. We have identified 20 hit genes that confer synthetic lethality in the absence of ARID1A or SMARCA4, respectively. Further validation efforts in an ARID1A knockout cell line showed a distinct functional family of three genes whose knockdown leads to a dramatic

decrease in cell viability in ARID1A mutant cells, whereas cells with wild type ARID1A are barely affected by the knockdown. The transcriptome of ARID1A knockout cells shows a significant deregulation of myc target genes which is even amplified by the knockdown of the three hit genes identified in the RNAi screen, pointing at a potential connection of the synthetic lethality and myc target gene expression.

87 De novo donor-specific HLA antibodies in kidney transplanted children

Rusai K. (1) Dworak,J.(1), Potemkin A. (1), Fischer G.(1), Csaicsich D. (1), Arbeiter K (1), Aufricht C. (1), Müller-Sacherer T. (1)

(1) Medical University Vienna, Austria, Department of Paediatrics and Adolescent Medicine; 2 Medical University Vienna, Austria, University Clinic for Blood Group Serology and Transfusion Medicine

Medical University Vienna, Austria, Department of Paediatrics and Adolescent Medicine johannadworak@hotmail.com

The significance of donor-specific HLA antibodies (DSA) has extensively been studied in adult transplanted patients. In the paediatric population, much less is known, in particular, on de novo DSA development, its impact on graft function and association with suboptimal immunosuppression. The aim of this study was to assess the development of de novo DSA in a prevalent cohort of 40 renal transplanted children and adolescents and to prospectively follow its association with clinical parameters, graft function and proteinuria for one year. Data was gathered retrospectively at the Medical University of Vienna, Austria Department of Paediatrics and Adolescent Medicine, Division of Paediatric Nephrology and Gastroenterology, 40 eligible renal transplanted children were included. HLA-Ab screening was performed in all patients in a cross-sectional way and laboratory data (estimated glomerular filtration rate (eGFR), proteinuria, calcineurin inhibitor trough level) were assessed retrospectively every 4th month one year before and prospectively one year after the date of antibody detection. At a median post-transplant time of 5 years, 17% of the patients had de novo DSA. All HLA-Ab were anti-HLA class II antibodies and persisted in 85% of the cases until the follow-up screening performed within one year. Basic clinical and laboratory parameters (age, gender, type of donation, HLA mismatch, post-transplant follow-up time and graft function) did not differ between DSA-negative and positive patients at the time of HLA-Ab screening. The changes of estimated glomerular filtration rate did not differ during the study period but there was a significantly higher increase in proteinuria in the DSA-positive patients during follow-up. Summarizing, our data demonstrates an overall prevalence of 17% of de novo DSA in a paediatric renal transplant cohort. During 12 months of prospective follow-up time we could demonstrate a significant impact of de novo DSA presence on proteinuria.

88 Proliferation and activation state of T cells in Multiple Sclerosis Lesions

Machado-Santos, J. (1), Saji, E. (2), Bauer, J. (3), Lassmann, H. (4)

Center for Brain Research, Vienna

joana.machadodossantos@meduniwien.ac.at

Multiple sclerosis (MS) is an autoimmune inflammatory CNS disease. It is well established that active demyelination and neurodegeneration in the MS brain is associated with inflammation, but the exact nature of the inflammatory response is currently undefined.Therefore, we aim to investigate the proliferation and activation state of the infiltrating T cells in the lesions of MS in comparison with other inflammatory CNS diseases.We analyzed formalin fixed paraffin embedded brain tissue from 23 MS patients, 1 neuromyelitis optica (NMO), 1 acute disseminated encephalomyelitis (ADEM), 7 Rasmussen´s encephalitis, 20 viral encephalitis, 21 stroke cases, 14 cases of Alzheimer´s and 10 healthy controls. We examined the expression of Ox40, NFAT, PD-1 and CCR5 for the activation and the expression of PCNA and MCM2 as proliferation markers by immunohistochemistry double staining with the T cell markers, CD3 and CD8, and the B cell marker,CD20.We observed high numbers of T cells, mainly CD8+ cells, in MS patients when compared with healthy controls, Alzheimer and Stroke. Interestingly, we only found B cells in high numbers in the perivascular areas of MS and NMO. We observed a significantly higher proliferation rate of CD3 and CD8 T cells in viral encephalitis diseases when compared with MS subjects, Stroke, Alzheimer´s and healthy controls. Regarding activation we found Ox40 expression on high numbers on T cells of NMO, but very low expression in MS. Conversely, the activation marker CCR5 was found to be highly expressed on lymphocytes of MS. These findings suggest that activated and proliferating T cells are more abundant in acute diseases like ADEM and viral encephalitis than in chronic lesions of MS. The high number of B cells present in the lesions of MS suggests a major role of these cells in the MS pathogenesis. The high number of CCR5+ cells in MS may have therapeutic implications since it could be targeted as an anti-inflammatory treatment using Maraviroc.

89 Role of arachidonic acid metabolite 12(S)-HETE in breast cancer lymph node metastasis

Chi Huu Nguyen Stefan Brenner, Serena Stadler, Walter Jaeger, Georg Krupitza

Department of Pharmacy and Diagnostics, University of Vienna, Clinical Institute of Pathology, Medical University of Vienna

nghchi86@yahoo.com.vn

Breast cancer is the most common cancer in women spreading mainly via lymph vessels. However, the mechanisms of tumor intravasation into the lymphatic vasculature are poorly understood. Current research is this filed mainly focuses on the molecular biology of tumor cells, yet little is known regarding the contribution of microenvironment such as lymphatic endothelial (LEC) vasculature. Therefore, we focused not only on pro-metastatic signaling of the tumor but also on that in LECs.Tumour spheroids secrete the endothelial retraction factor 12(S)-HETE and this repels LECs thereby generating disintegrated areas in the LEC monolayer so called “circular chemorepellent induced defects” (CCIDs), through which tumor emboli can intravasate. Along with CCID assay, molecular and pharmacological inhibitors approach, western blotting, q-PCR analysis, calcium influx, and 12(S)-HETE synthesis assay were used to elucidate the mechanisms.We demonstrate that in MDA-MB231 breast cancer cells, which do not express ALOX12/15 as the major 12(S)-HETE producing enzymes, cytochrome P450 isoenzyme 1A1 (CYP1A1) alternatively synthesizes 12(S)-HETE. CYP1A1 itself is under direct control of arylhydrocarbon receptor (AhR) and both polypeptides are known to be over-expressed in various cancer types which qualifies them as potential targets for therapeutic intervention. In LECs 12(S)-HETE activates the mobility protein MLC2 and cell migration (as a prerequisite for CCID formation) through its receptor GPR31 and the downstream signal transducers RhoA and ROCK. In addition, 12(S)-HETE induces MLC2 and migration of LECs through triggering the release of intracellular Ca2+ and the subsequent activation of the calmodulin kinase isotype MYLK, which is independent of GPR31.In conclusion, the many steps within these signalling pathways provide potential targets for therapeutic intervention.

90 Neural Circuit Mechanisms Underlying the Anxiolytic Effect of Benzodiazepines

Griessner, J. (1, 3), Pasieka, M. (2), Boehm, V. (3), Pliota, P. (3), Kargl, D. (3), Haubensak, W. (3)

(1) Medical University of Vienna, Austria, (2) Vienna Biocenter Core Facilities GmbH, Vienna, Austria (3) Research Institute of Molecular Pathology, Vienna, Austria

johannes.griessner@imp.ac.at

Benzodiazepines (BZDs) have been in clinical use for decades to treat fear and anxiety, and the molecular mechanisms by which they change the activity of single neurons are now well understood. However, little is known about which neuronal circuits they modulate in the brain, and how this, in turn, leads to their anxiolytic effect. Here, we used circuit genetic manipulations and pharmacology in mouse models for anxiety to address this question. To this end, we performed a limbic system wide c-fos activity screen to identify hot-spots of neuronal

modulation after BZD administration. We found that BZD treatment significantly increases the number of c-fos expressing neurons in the lateral central amygdala (CeL), and that this activity correlates with behavioral measures of their anxiolytic effect in the elevated plus maze (EPM). The CeL is composed of two antagonistic inhibitory populations, marked by the expression of either PKC-[delta] or Somatostatin/CRH. This local inhibitory circuitry gates amygdala output and fear when the PKC-[delta][up+] neurons are active. We found that BZD administration increased c-fos activity predominantly in PKC-[delta][up+] cells, indicating that BZDs might act, at least in part, by modulating neuronal activity of this population. To test this directly, we used pharmacogenetic methods to specifically modulate CeL PKC-[delta][up+] neurons:While driving their activity was sufficient to mimic BZD-induced anxiolysis in the EPM, inhibition of CeL PKC-[delta][up+] neurons completely abolished this BZD effect. These data strongly implicate CeL PKC-[delta][up+] neurons in the anxiolytic action of BZDs, through inhibitory gating of stress signals in the central amygdala.Taken together, we provide a circuit mechanistic framework for the BZD anxiolytic effect which might help the development of novel therapeutics for the treatment of anxiety disorders in the future.

91 Selective ligand design for vasopressin GPCR subtypes using invertebrate peptide homologs

Maria Giulia Di Giglio (1), Markus Muttenthaler (2), Christian W. Gruber (1),(3)

(1) Center for Physiology and Pharmacology, Medical University of Vienna, (2) Institute for Molecular Bioscience, (3) School of Biomedical Sciences, The University of Queensland, Australia

maria.digiglio@medunwien.ac.at

The neurohypophysial hormones vasopressin (AVP) and oxytocin (OT) are crucial for a wide range of functions, including water retention, cardiovascular homeostasis and social behavior. In humans AVP and OT mediate their biological actions through specific G-protein coupled receptors (GPCRs), three distinct receptor subtypes V1a, V1b, V2 and one OT receptor. The development of novel GPCR targeting peptide drugs is often hampered by sequence homology of the ligands and structural similarity of the different receptor subtypes causing non-specific and unwanted effects. Our aim is to harness the molecular conservation of GPCRs for the design of novel peptide ligands with improved selectivity and molecular signalling properties. We identified the precursor sequence of the peptide inotocin (INT) in several ant species, then cloned and expressed the cognate receptor in mammalian cell culture. INT is an invertebrate structural homolog of AVP. The affinity and potency of INT were measured and compared to all four AVP and OT receptor subtypes, by means of radioligand binding and quantitative second messenger experiments. To increase the stability and improve the selectivity, D-amino acid scans of the native insect peptide were conducted. [D-Arg8]INT exhibited a novel agonist/antagonist switch

at the V1a receptor, based on the interaction of the arginine side chain with a non-conserved residue in the V1a receptor triggering an ‘on/off’ switch. In silico GPCR modelling has pointed out residues in the human receptor sequence, which might be responsible for the observed effects.Overall, natural products play a pivotal role in modern drug discovery and they continue to provide innovative lead compounds for pharmacological analysis. As a proof-of-concept, we demonstrate that nature-derived peptides can be used as pharmacological tools to study structure-activity relationship and be used as templates to synthesize optimized peptide ligands of human GPCRs.

92 PERITONEAL DIALYSIS FLUID CAUSE INADEQUATE ACTIVATION OF HSF1 BY RELEVANT STRESSORS A NOVEL PATHOMECHANISM?

Herzog, R. (1, 2, 3) Kratochwill, K (1, 3), Lederhuber, H. (1), Kuster, L. (1, 2), Heindl-Rusai, K. (1), Salzer, E. (1), Bidmon, B. (1), Lichtenauer, A. (1,2), Aufricht, C. (1)

(1) Medical University of Vienna, Department of Pediatric and Adolescent Medicine, Pediatric Nephrology, (2) Zytoprotec GmbH, Vienna, (3) Medical University of Vienna - Christian Doppler Laboratory for Molecular Stress Research in Peritoneal Dialysis

rebecca.herzog@meduniwien.ac.at

Peritoneal dialysis (PD) is an alternative to hemodialysis. Chronic exposure to PD-fluid (PDF) causes injury of mesothelial cells (MC) but also induces cytoprotective mechanisms. Recent studies suggest that PDF blocks the heat shock response (HSR), one of the evolutionary most important stress responses. The resultant increased vulnerability of the MC could lead to progressing fibrosis of the peritoneal membrane. The aim is to identify the molecular mechanisms leading to the PDF-induced inadequate HSR on the level of the heat shock transcription factor 1 (HSF1).The induction of the HSR in human MC was analyzed using combined models of PDF-exposure and heat stress as the gold standard. In addition single cytotoxic components of PDF (glucose degradations products (GDP) and acidosis) were investigated. The status of HSF1 activation, cellular Hsp72 expression, the stress-proteome and viability of the MC were analyzed. HSF1 regulation was analyzed by use of nuclear shift analysis, the phosphorylation status, DNA-binding capacity and Hsp72 induction.Compared to heat, PDF leads to increased lethality but decreased Hsp72 expression. A concurrent blockage of the nuclear shift, phosphorylation and DNA-binding of HSF1 with reduced activity of the promotor was found. The inadequate HSF1 activation could be unblocked by a neutral pH or PDF without GDPs. The HSF1 blocking caused by the acidosis was associated with activation of GSK-3β, while the GDPs directly interfered with HSF1 promotor activity.The PDF-mediated inadequate induction of the cellular HSR represents a new pathomechanism in PD. Our results demonstrate that the cytotoxic factors such as acidosis and GDPs of PDF lead to a HSF1 block via different molecular mechanisms resulting in a reduction of the HSR and increased

vulnerability of MC exposed to PDF. In further studies the role of post-translational modifications of HSF1 and their influence on the regulation of the HSR in PD will be analyzed.

93 A framework for allosteric activation of secondary active transport

Li, Y. (1), Mayer, FP. (1), Hasenhuetl, PS. (1), Schicker, K. (1), Sitte, HH. (1), Freissmuth, M. (1), Sandtner, W. (1)

Institute of Pharmacology, Medical University of Vienna

yang.li@meduniwien.ac.at

We recently showed that Zn[up2+] is an allosteric activator of substrate transport by the human dopamine transporter (DAT). The ability to stimulate a secondary active transporter is a desired ligand quality (i.e. in the treatment of diseases caused by loss of function mutants). However, to date no drug is available that was described to work this way. In this study, we decided to further explore this subject by taking advantage of DAT and the fact that it is endowed with an endogenous binding site for Zn[up2+]. This site can accommodate the divalent cation Ni[up2+], which we identified as a stimulator of substrate uptake. This extended our repertoire of testable modulators and helped us pinpoint novel aspects in the stimulation of transport by such ligands. These accelerate the transport rate by displaying differing affinities for conformational intermediates adopted in the transport cycle. In this study we show that activators of substrate transport do not only differ in their affinities for the outward and inward facing conformations (as previously described) but also in their affinities for the apo and substrate bound conformations. Moreover, we demonstrate that preferred binding of the apo-conformations can render their stimulatory action less sensitive to inhibition by high intracellular Na[up+] concentrations [Na[up+]]i. We show that augmented stability against a rise in [Na[up+]]i is achieved by conversion of a higher affinity/lower capacity into a lower affinity/higher capacity transporter.

94 Functional analysis of two Austrian RYR1 variants linked to malignant hyperthermia

Mashaghi, M.(1) Mann, A.(1), Weigl, L.(1)

Medical University of Vienna, Medical University of Vienna

maryam.mashaghitabari@meduniwien.ac.at

The ryanodine receptor 1 (RYR1) and other proteins such as the dihydropyridine receptor (DHPR) regulate muscle contraction by controlling the release of calcium. Malignant hyperthermia (MH) is a pharmacologic disorder of the skeletal muscle, where patients suffer from a hypermetabolic syndrome when volatile anaesthetics such as halothane are administered. In most cases, the metabolic changes are caused by dysregulation of skeletal muscle Ca2+ homeostasis due to mutations in the RYR1. This intracellular Ca2+ release channel resides in the sarcoplasmic reticulum and controls the flux of Ca2+ ions from the SR into the cytoplasm. Over 400 variants potentially linked to MH have been identified in the RYR-1 gene located on chromosome 19q13.1, but currently only 35 of them are considered causal for MH. Since the current MH diagnosis relies on the invasive in vitro contracture test (IVCT) of biopsied muscle, it is desirable to establish a genetic test. Today, the genetic test can only be performed for mutations recognised as causative by the European Malignant Hyperthermia Group (EMHG). To apply a genetic test as a routine test for MH suspicious individuals, it is necessary to prove causality to as many mutations as possible. Here we describe the functional analysis of 2 Austrian variants that were found in 2 unrelated malignant hyperthermia susceptible families. However, the frequency of these variants is too low to be validated as causative by population genetics. Therefore, the functional properties of the mutated RYR1 were analysed by cloning and expression of these variants into different eukaryotic cell lines. The Ca2+ release properties of mutated RYR1 are compared to the wild type with Ca2+ imaging to be able to confirm a possible causality for MH of these variants. The expression of the RYR1 is confirmed by western blot analysis. With this work, we hope to be able to add these variants to the list of causative MH mutations.

95 The role of Phl p 5-specific IgG antibodies for allergen presentation

Gabriela Sánchez Acosta Sánchez Acosta G.(1), Faustmann S.(1), Focke-Tejkl M.(1), Wortmann J.(2), Keller W.(2), Bohle B.(1)

(1) Center of Pathophysiology, Infectiology and Immunology, Department of Pathophysiology and Allergy Research, Medical University of Vienna, Austria, (2)Institute for Molecular Biosciences, Karl Franzens University, Graz, Austria

gabriela.sanchezacosta@meduniwien.ac.at

Background: Allergen-specific immunotherapy (SIT) is based on the administration of appropriate concentrations of allergen extracts. A beneficial response in patients has been associated with high productions of IgG4 and IgG1 antibodies, which compete with IgE for allergen binding. However, allergen-IgG complexes can also bind to FCγ-receptors expressed on the surface of antigen-presenting cells (APC). This cross-linking may thereby increase allergen-uptake and eventually the number of HLA-peptide-complexes on the surface of these cells which may drive the resulting T cell response towards Th1.Method and results: We will study the

effects on the T cell level induced by the decrease of the IgE/IgG ratio using the major grass pollen allergen, Phl p 5. This recombinant allergen was expressed and characterized and will be incubated with human Phl p 5-specific monoclonal IgG1, IgG4 and IgE antibodies with identical paratop. In addition, sera from AIT-treated patients containing high levels of Phl p 5-specific IgG will be used. Professional APCs will be isolated from whole blood samples in order to compare surface binding, internalization and processing of IgE-, IgG-bound and unbound Phl p 5. To asses proliferative and cytokine responses, Phl p 5 specific T cell lines and T cell clones will be produced and stimulated with APCs pulsed with antibody-loaded and unloaded Phl p 5. Finally, these latter aspects will also be investigated by using naïve T cells. Together, this data will show if AIT-induced IgG antibodies may not only block IgE-mediated effects but also modulate allergen-specific T cell responses during the therapy.

96 Do SNPs in VKORC1 have a direct impact on systemic arterial blood pressure?

Sara Omid Adelheid Panzenböck, Christian Gerges, Max-Paul Winter, Mario Gerges, Christine Mannhalter, Irene M. Lang

Medical University of Vienna, Department of Internal Medicine II, Division of Cardiology, Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University Vienna, Vienna, Austria

n1105892@students.meduniwien.ac.at

BACKGROUND AND AIM: VKORC1 is a membrane-intrinsic enzyme localized in the rough endoplasmic reticulum. It is responsible for reduction of inactive vitamin K epoxide to the active vitamin K hydroquinone and the rate limiting step in the vitamin K cycle. VKORC1 polymorphisms affect warfarin dose response and are associated with vessel calcification, aortic aneurysms and stroke and thus may represent a novel genetic marker for cardiovascular disease. In the present study we aimed to investigate the effect of two VKORC1 polymorphisms [(3730) G>A and (-1639) G>A] on hypertension and other clinical parameters.METHODS AND RESULTS:We conduct a prospective observational study including patients who undergo elective coronary angiography at the General Hospital of Vienna. Data acquisition is performed by a systematic exploration of the centralized patient management system of the Vienna General Hospital (AKIM). In a substudy of 213 patients with two consecutive coronary angiographies at least one year apart we analyzed the effect of VKORC1 polymorphism on the probability to develop hypertension. Individuals carrying the VKORC1 (-1639) AA variant leading to a decreased VKORC1 activity showed a tendency of elevated systolic blood pressure (p=0.079) and significantly elevated pulse pressure ($p \leq 0.04$) at the time point of the second coronary angiography. Carriers of the VKORC1 (3730) AA variant leading to an increased VKORC1 activity showed significantly reduced mean blood pressure ($p \leq 0.04$) and a tendency of reduced systolic and diastolic blood pressure (p=0.93 and 0.78 respectively).CONCLUSIONS: Both

VKORC1 SNPs show a correlation with systemic arterial blood pressure in a substudy of 213 patients with two consecutive coronary angiographies. This suggests a role of VKORC1 in blood pressure regulation most likely through pathways involving vitamin K epoxide reductase and calcium binding proteins like matrix gla (MGP).

97 Investigation of factors underlying KP1339-hypersensitivity

Alte, B. (1, 2) Pirker, C. (1); Thomas Mohr, T. (1); Kuhn, PS. (2); Kryeziu, K. (1); Körner, W. (4); Hermann, G. (3); Köllensperger, G. (3); Bernhard K. Keppler (2); Heffeter, P. (1); Berger, W. (1)

(1) Medical University of Vienna, Institute of Cancer Research; (2) University of Vienna, Institute of Inorganic Chemistry; (3) University of Vienna, Department of Analytical Chemistry; (4) University of Vienna, Department of Environmental Geosciences

beatrix.alte@univie.ac.at

The ruthenium-based compound KP1339 (sodium salt of trans-(tetrachlorobis(1H-indazole)ruthenate(III)) is already known for its promising anticancer activity. Having successfully completed a clinical phase I trial, research now focuses on the mechanism underlying (hyper-)sensitivity to KP1339 treatment. In a screen using 25 cell lines of diverse tumor types, a small subgroup with exceptional sensitivity was identified and selected for further analysis. This panel of cell lines was complemented with three cell lines displaying intrinsic resistance. As a first approach, the panel was analysed concerning prevailing sensitivity factors: GSH levels, accumulation of and hypersensitivity to ruthenium and indazole representing the building blocks of KP1339. Noteably, no significant differences were found, suggesting the existence of target(s) especially inside hypersensitive cell models instead of trivial accumulated dose effects. To help identify these possible targets, the biochemical response of cells during a short time exposure to KP1339 was analysed in detail. Therefore, gene expression arrays were performed with mRNA isolated from cells of the selected panel that were either untreated or treated with KP1339 for 3 h and 6 h. Subsequent bioinformatic analysis indicated that while hypersensitive cell lines activated pathways that respond to chemical stimuli, in intrinsically resistant ones preferentially genes involved in pathways controlling cell cycle, DNA repair and metabolism were found. Adjacent 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). Taken together, this study indicates that the anticancer activity of KP1339 is based on a specific mode of action rather than on conventional chemical poisoning of the cells.

98 Tick borne encephalitis (TBE) booster in allergic patients: effects of allergy and SIT treatment on humoral and cellular immune responses

Garner-Spitzer, E. (1)Hofer, M.(1), Seidl-Friedrich, C.(1), Kinaciyan, T.(2), Kundi, M.(3), Zlabinger, G. (4) Wiedermann, U.(1)

(1) Institute for Specific Prophylaxis &Tropical Medicine, Center for Pathophysiology, Infectiology & Immunology; Medical University of Vienna, (2) Department of Dermatology, DIAID, Medical University of Vienna, (3) Institute of Immunology, Medical University of Vienna, (4) Institute of Public Health, Medical University of Vienna

erika.garner-spitzer@meduniwien.ac.at

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/TGFß and shift to Th1-profile. In the current clinical trial we investigated whether responsiveness to routine vaccines is altered by allergy: allergics -/+ SIT and controls received a booster vaccination for tick-borne encephalitis (TBE). Immune-responses were evaluated via specific Ab-titers, IgG subclasses, cytokine-profiles and naïve/memory/regulatory subsets of lymphocytes. We report that titer profiles and magnitude of humoral responses to TBE booster were comparable between allergics -/+ SIT and controls. Total and TBE-specific IgG subclasses and in-vitro cytokines showed Th2 polarization on humoral/cellular level in allergics before vaccination, which was “reverted” on humoral level 1 month post booster in both allergic groups. Allergy and SIT lead to altered distributions of B- and T-cell subsets, e. g. more late-differentiated CD4-memory T-cells and a high increase of plasmablasts post booster in allergics. SIT patients had more CD4 T-cells, expanded memory subsets of CD4 / CD8 T-cells and B-cells as well as increased plasmablasts and FOXP3+T-regs. Allergics -/+ SIT had increased expression of FcεRII (CD23) on B-cells. No enhanced reactogenicity was observed in allergic groups and no exacerbation of allergic symptoms due to vaccination was reported. Alterations of the immune system due to allergy/SIT are reflected in substantial changes of T- and B-cell sub-populations, however booster vaccine responses are not impaired in allergics -/+ SIT. Vaccination seems to positively influence Th1/Th2 dichotomy leading to “depolarization” of the allergy bias. Vaccination is not linked to increased reactogenicity and exacerbation of allergic symptoms, thus there is no indication to refrain from routine vaccines in allergic patients.

99 Employing CRISPR/Cas9 for fluorescent tagging of endogenous proteins to investigate their dynamic interactions in live cells

Moser, B. Hochreiter, B., Schmid, J.A.

Medical University of Vienna

bernhard.a.moser@meduniwien.ac.at

Most of the protein interactions have been identified qualitatively by screening techniques and biochemical analysis in vitro under non-physiological conditions. However, a more detailed understanding of these interactions and the dynamic dissociation and re-association reactions within the complex environment of live cells is still lacking. Live cell experiments are often done with fluorescent proteins attached to the protein of interest after transfection of respective expression plasmids. Interactions can then be determined by using spectrally distinct fluorescent tags and the quantum physical phenomenon of fluorescence resonance energy transfer (FRET). We developed a technique combining FRET microscopy with FRAP (fluorescence recovery after photobleaching) and have evidence that the fluorescence recovery kinetics provide information on the k-off rate of the interaction and thus on the half live of a protein complex. However, the normal transient transfection approach suffers from the drawback that the interaction partners are expressed at unnaturally high levels. We intend to surpass these issues using genome-editing methods based on the CRISPR/Cas9 technology in order to label endogenous signaling molecules in live cells and furthermore maintaining the natural promoter, as well as 5' and 3' UTR regulatory regions. Our novel FRET-FRAP microscopy technique will then be applied to fluorescently tagged molecules at their physiological states. Moreover, we will also employ the CRISPR/Cas9 technology to attach a genetically engineered Biotin ligase to a protein of interest, which will then allow labeling of binding partners with biotin and subsequent identification by means of mass spectrometry. This will enable us to elucidate the complex interaction network of signaling molecules at physiological expression levels We are convinced that our work will lead to a better understanding of various molecular protein- and signaling networks.

100 Influence of IL-9 skewing conditions on skin resident T cells

Kienzl, P. (1), Polacek R. (1), Hagenbach P. (1), Gassner T. (1), Tajpara P. (1), Gschwandtner M. (2), Mildner M. (2), Elbe-Bürger A. (1)

(1) Medical University of Vienna, Laboratory of Cellular and Molecular Immunobiology of The Skin, Division of Immunology, Allergy and Infectious Diseases, Department of Dermatology, Vienna, Austria, (2) Medical University of Vienna, Research Division of Biology and Pathobiology of The Skin, Department of Dermatology, Vienna, Austria

philip.kienzl@meduniwien.ac.at

TH9 cells are a recently characterized T cell subset, characterized by the production of IL-9 and can be detected primarily within the skin-homing TH population. Adapting a skin T cell culture system we investigated the effects of IL-9 skewing cytokines on skin resident T cells. Skin biopsies from healthy donors were cultured on cell foam matrices (grids) either in the presence

of IL-2 and IL-15 (standard condition) or IL-2, IL-4 and TGF-beta (TH9-promoting condition). Both culture conditions favored the proliferation of CD3+ T cells (90-98%). Standard conditions consistently yielded a higher number of T cells (1.2x10[up6]/grid) as compared to TH9-promoting conditions (0.8x10[up6]/grid). We found significantly more Ki-67+ T cells at standard conditions as compared to TH9-promoting conditions indicating that cell division contributes to overall cell counts. The number of CD4+ T cells was higher in cultures at TH9-promoting conditions (40.8%) compared to standard conditions (24.3%) after 4 weeks. IL-9 producing T cells emerged at week 2 (6%) and steadily increased until week 5 (27%) and interestingly, more CD8+ T cells produced IL-9 compared to CD4+ T cells (32% vs. 3.5%, respectively) when cultured at TH9-promoting conditions. Under standard conditions no IL-9 producing T cells were identified at all investigated time points. CLA could be readily identified on T cells when skin was cultured on grids but not on T cells without grids. Additionally, we employed a human skin equivalent model to study effects of IL-9 on keratinocyte differentiation because TH9 cells are involved in psoriasis and atopic dermatitis. IL-9 treatment led to the downregulation of keratin 10 and involucrin in preliminary experiments. In conclusion, IL-2, IL-4 and TGF-beta promote the preferential development of IL-9 producing T cells from healthy human skin. Furthermore, IL-9 might be involved in the cornification process.

101 Age dependent changes of the knee joint using the example of the intercondylar notch, the meniscofemoral ligaments and the posterior cruciate ligament

Hirtler, L., Röhrich, S., Kainberger, F.

Medical University of Vienna, Department of Biomedical Imaging and Image-guided Therapy

lena.hirtler@meduniwien.ac.at

Aims: The morphology of the intercondylar notch, the meniscofemoral ligaments (MFLs) and the posterior cruciate ligament change continuously during life. This will be shown in this study and will be discussed in clinical context.Material and methods:Retrospective study using 3T knee-MRIs of 342 patients. The selection of the patients was based on exclusion criteria, the subdivision in groups was based on their respective age. The specific groups were chosen to represent physical growth, respectively the changes of adulthood and later in life. The intercondylar fossa was measured and its shape assessed, incidences, lengths and cross sectional area of the MFLs and their interaction with the posterior cruciate ligament were evaluated in different stages of life and tested for gender differences.Results:The intercondylar notch is subjected to continual changes, which influence its width of 16.2 to 18.6 mm throughout life. A change of shape from A-shape to Ω-shape later in life was observed. The growth in length of the MFLs stops before the age of 11, though the cross-sectional area of them increases further. The cross-sectional area of the posterior cruciate ligament is significantly larger if the posterior MFL is missing.Conclusion:The term of intercondylar space was defined as an anatomical space

between the femoral condyles including all ligaments situated within. The femur is subjected to lifelong three-dimensional changes, which not only affect the proximal part or the femoral torsion, but also its distal part. The MFLs of the human knee are necessary structures, which work synergistically with other elements of the knee joint, especially with the posterior cruciate ligament. The missing of a MFL is balanced by above average growth of the other existing MFL or of the posterior cruciate ligament.

102 The TYK2-STAT1 pathway in aggressive T-Cell Lymphoma: A new therapeutic intervention site?

Prutsch, N.(1), Sanda, T. (2), Wu, L. (3), Turner, S. (4) , Jaeger, U. (5), Staber, P. (5), Mueller, M. (6), Kenner, L. (1,6), Merkel, O. (1)

Did not appear

(1) Institute of experimental Pathology, (2) Cancer Science Institute of Singapore, National University of Singapore; (3) Department of Immunology, Genentech Inc., 1 DNA Way, South San Francisco, CA 94080, United States; (4) Division of Molecular Histopathology, Department of Pathology, University of Cambridge, Cambridge, UK; (5) Department of Medicine I, Clinical Division of Hematology and Hemostaseology and the Comprehensive Cancer Center, Medical University of Vienna, Vienna, Austria; (6) Institute of Animal Breeding and Genetics, University of Veterinary Medicine, Vienna, Austria

nicole.prutsch@meduniwien.ac.at

Whole genome sequencing of T-cell lymphomas has revealed mutations in members of the Januskinase family (JAK1, JAK2, JAK3, TYK2), known to have essential functions in cytokine signalling, motility and proliferation. Targeting Januskinases has opened new therapeutic intervention sites in cancer and autoimmune diseases. Tyrosinekinase 2 (TYK2) the first JAK kinase to be identified has important roles in inflammation and anti tumor immunity. Here we report pathway dependence of TYK2 in anaplastic large cell lymphoma, a CD30 positive, aggressive Non-Hodgkin T-cell lymphoma. The importance of TYK2 is underlined by the recent description of TYK2-fusions driving oncogenic signalling in ALCL. Interestingly, using RNAseq data of 23 primary ALCL cases TYK2 expression was generally high as compared to PBMC independent of the presence of TYK2-fusions, suggesting active TYK2 signalling in large fraction of patients. Ablating TYK2 in ALCL cell lines, using lentivirally transduced shRNAs as well as CRISPR/CAS9 technology, led to rapid cell death induction.To test the therapeutic relevance of our findings ALCL cell lines and PBMCs were treated with small molecule inhibitors of TYK2 (TYK2#2,Bayer-18) leading to apoptosis induction exclusively in tumor cells. Immunoblotting and shRNA mediated knockdown of potential downstream targets of TYK2 revealed an essential function for STAT1 in ALCL cell survival.Flow cytometric analyses revealed active regulation of PD-L1 in TYK2 depleted cells. Tumor intrinsic and immune-ceckpoint relevant consequences of TYK2 knockout are currently being assessed using

an ALCL mouse model with T-cell specific TYK2 knock-out and experiments employing T-cell activation reporter cell lines with and without PD-1 expression.

103 Identification of genetic deletions interacting with ETV6-RUNX1 fusion gene in B-ALL

Aigner Petra (1), Horvath, J. (1,2), Zuber, J. (3), Panzer-Grümayer, R. (4), Stoiber-Sakaguchi, D. (1,2)

(1) Ludwig Boltzmann Institute for Cancer Research, Vienna, Austria, (2) Institute of Pharmacology, Center for Physiology and Pharmacology, Medical University of Vienna, Vienna, Austria, (3) Research Institute of Molecular Pathology (IMP), Dr. Bohr Gasse 7, Vienna 1030, Austria, (4) Children's Cancer Research Institute and St. Anna Kinderspital, Department of Paediatrics, Medical University, Vienna, Austria

Petra.Aigner@lbicr.lbg.ac.at

Acute lymphoblastic leukaemia (ALL) is the most common cause of death by disease in children younger than 15 years. Chromosomal alterations and genetic abnormalities can be found in around 75% of all childhood ALL cases. The most common genetic alteration found in B-ALL (up to 25%) is the translocation t(12;21) encoding the fusion gene ETV6-RUNX1. Although ETV6-RUNX1 plays a major role in the progression of B-ALL, it has been shown that it is insufficient to induce overt B cell leukaemia, neither in patients nor in mouse models. Thus, ETV6-RUNX1 is generally associated with additional mutations and deletions in genes playing crucial roles in cell cycle control, (B-) lymphocyte development and cell differentiation. Among others ETV6, PAX5, IKZF1, BTG1, CDKN2A/B and RB1 have been shown to be frequently deleted or mutated in ETV6-RUNX1-carrying B-ALL cases. However, it is still unknown which additional mutations are imperative in combination with ETV6-RUNX1 for developing overt B-ALL. Therefore, our aim is to identify secondary deletions in an ETV6-RUNX1-based mouse model, leading to the development of a B-ALL-like phenotype. In order to achieve that, we use a shRNA library to knockdown several potential genes in murine ETV6-RUNX1-expressing bone marrow derived B cells. Cells are then transplanted into immunodeficient NOD-SCID-IL2Rcgnull (NSG) mice and monitored for hematopoietic parameters in expectation of a B cell leukaemia-like phenotype. Conclusively, leukemic cells will be isolated and evaluated via next generation sequencing (NGS) to identify differences compared to the pre-transplantation status. Those experiments are supposed to reveal a combination of genes, whose (partial) loss in combination with ETV6-RUNX1 can cause a stable B-ALL-like cancer in mice. Such a mouse model can serve as an important base for further research on the detailed role of single genes in the development of ETV6-RUNX1-positive B-ALL and their interaction.

104 Oligomerization of human dopamine transporter (hDAT)

Kumaresan Jayaraman, Harald H Sitte, Thomas Stockner

Institute of Pharmacology, Medical University of Vienna

kumaresan.jayaraman@meduniwien.ac.at

105 PAX6 as an Effector of ATG7-Dependent Autophagy in UVA-Stressed Limbal Stem Cells

Laggner, M. (1), Pollreisz, A.(2), Schmidinger, G.(3), Schmidt-Erfurth, U.(4), Chen, Y.-T.(5)

Department of Ophthalmology & Optometry

maria.laggner@meduniwien.ac.at

106 STAT1 promotes immune escape of intestinal cancer

Jasmin Svinka (1), Ilija Crncec (1), Michaela Schlederer (2,3), Martin Filipits (1), Lukas Kenner (2,3), Emilio Casanova (2,4), Mathias Müller (5), Birgit Strobl (5), Gerwin Heller (6), Robert Eferl (1)

(1) Medical University Vienna & Comprehensive Cancer Center (CCC), Institute for Cancer Research, Vienna, Austria (2) Ludwig Boltzmann Institute for Cancer Research LBICR, Vienna, Austria (3) Institute of Clinical Pathology, Medical University of Vienna, Austria (4) Institute for Pharmacology and Toxicology, University of Veterinary Medicine Vienna, Austria (5) Institute of Animal Breeding and Genetics, University of Veterinary Medicine Vienna, Austria (6) Medical University Vienna & Comprehensive Cancer Center (CCC), Clinical Division of Oncology, Vienna, Austria

jasmin.svinka@meduniwien.ac.at

107 The intracellular gating network of the human dopamine transporter

Morley, Alexander (1), Mayer, Felix P.(1), Sitte, Harald H. (1), Stockner, Thomas (1)

Medical University of Vienna, Institute of Pharmacology

alexander.morley@meduniwien.ac.at

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 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 effect of Zinc on mutants were investigated through zinc chloride inhibition of [3H]-MPP+ uptake in HEK293 cells expressing hDAT . The effect of zinc on substrate efflux was determined through loading of [3H]-MPP+ into HEK293 cells expressing hDAT before being challenged amphetamine and/or zinc chloride. Transport activity of hDAT with mutations present in the intracellular hydrophobic lid 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, but mutations that partially or fully remove this lock have been found to reduce amphetamine-mediated efflux. In order to further investigate the conformational effects of mutations, substrates and inhibitors on the conformational cycle, molecular dynamics simulations of hDAT are being conducted. Homology models were generated from the drosophila dopamine transporter (dDAT, PDB:4XP1) with modeller 9.16. Full-atomistic molecular dynamics simulations are being conducted using Gromacs 5.1.2 with an Amber99sb-ildn forcefield.

108 A COMPARISON OF THE β-ADRENERGIC RECEPTOR ANTAGONISTS LANDIOLOL AND ESMOLOL: RECEPTOR SELECTIVITY, PARTIAL AGONISM AND PHARMACOCHAPERONING ACTIONS

Nasrollahi-Shirazi, S. (1), Sucic, S. (1), Yang, Q. (1), Nanoff, C. (1), Freissmuth, M. (1)

Institute of Pharmacology, Center of Physiology and Pharmacology, Medical University of Vienna

shahrooz.nasrollahishirazi@meduniwien.ac.at

Blockage of β1[beta down1]-adrenergic receptors is one of the most effective treatments in cardiovascular medicine. Esmolol was introduced some three decades ago as a short-acting β1-selective antagonist. Landiolol is a more recent addition. Here we compared the two compounds for their selectivity for β1-adrenergic receptors over β2[beta down2]-adrenergic receptors, partial agonistic activity, signaling bias and pharmacochaperoning action by using HEK293 cell lines, which heterologously expressed each human receptor subtype. The affinity of landiolol for β1-adrenergic receptors and β2-adrenergic receptors was higher and lower than that of esmolol,

respectively, resulting in an improved selectivity (216-fold vs. 30-fold). The principal metabolite of landiolol (M1) was also β1-selective, but its affinity was very low. Hence it is unlikely to contribute to the action of landiolol in vivo. Both, landiolol and esmolol caused a very modest rise in cAMP (cyclic adenosine monophosphate) levels but a robust increase in the phosphorylation of extracellular signal regulated kinase-1 and 2 (ERK-1 &-2) indicating that the two drugs exerted partial agonist activity with a signaling bias. If cells were incubated for ≥[>=] 24 h in the presence of ≥ 1 μ[my]M esmolol, the levels of β1-adrenergic – but not of β2-adrenergic - receptors increased. This effect was contingent on export of the β1-receptor from endoplasmic reticulum and was not seen in the presence of landiolol. Based on these observations, we conclude that landiolol offers the advantage of (i) improved selectivity and (ii) the absence of pharmacochaperoning activity, which sensitizes cells to rebound effects upon drug discontinuation.Keywords: landiolol, esmolol, pharmacochaperoning, β-adrenergic receptors, biased agonism.

109 Quantification of nitric oxide in blood and tissue using endogenous nitric oxide trapping molecules

Dumitrescu, S. (1), Meszaros, A. (1,2), Puchner, S. (1), Weidinger, A. (1), Boros, M. (2), Redl, H. (1), Kozlov, A. (1)

(1) Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, (2) Institute of Surgical Research - University of Szeged

n0501827@students.meduniwien.ac.at

Nitric oxide (NO) is a powerful signalling molecule responsible for the regulation of a number of physiological and pathophysiological processes. Currently, the most sensitive method for the measurement of NO in biological tissues is electron paramagnetic resonance (EPR) spectroscopy, a method which in case of NO detection is based on the interaction of NO with exogenous NO-traps. However, such traps are potentially harmful and cannot be used in clinical settings. Our aim was to develop a detection method specific for NO in biological samples using NO complexes that occur naturally in blood (mononitrosyl-hemoglobin, NO-Hb) and tissue (dinitrosyl-iron, NO-Fe). To identify the signals corresponding to NO-Hb and NO-Fe complexes within EPR spectra, we analysed EPR spectra of rat liver samples, extracted 16 hours after induction of a systemic inflammatory response of different severity (0 to 8 mg/kg lipopolysaccharide, LPS). Endogenously occurring NO-Hb and NO-Fe signals were quantified based on specific calibration samples, which were prepared from erythrocyte suspensions and liver homogenates by incubation with distinct NaNO2 concentrations.Both NO-Hb and NO-Fe complexes were detected in liver tissue extracted from animals injected with LPS, whereas in erythrocyte-free liver calibration samples only NO-Fe (g = 2.042) was detected. In contrast, blood withdrawn from animals injected with LPS and erythrocyte suspensions showed only NO-Hb complexes (g = 2.075). NO-Hb and NO-Fe signals measured in calibration samples showed a

linear increase and a strong correlation with NaNO2 concentration.The method presented here enables to quantify extracellular and intracellular levels of NO in a single frozen liver biopsy without pre-treatment with potentially harmful exogenous NO-traps. Furthermore, it enables to allocate NO-Hb and NO-Fe signals to their specific intra- and extracellular compartments of liver.

110 Activated STAT5 Drives Peripheral T Cell Lymphomas

Maurer, B. (1, 2),Nivarthi, H. (3), Prchal-Murphy, M. (4), Wingelhofer, B. (1, 2), Chen, D. (3), Winkler, S. (1), Schlederer, M. (1, 5), Pham, H. (1, 2), Kenner, L. (1, 5), Sexl, V. (4), Kolbe, T. (6, 7) Kralovics, R. (3), Müller, M. (2), Rülicke, T. (8), Moriggl, R. (1, 2, 9)

(1) Ludwig Boltzmann Institute for Cancer Research, (2) Institute of Animal Breeding and Genetics, University of Veterinary Medicine Vienna, (3) CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, (4) Institute of Pharmacology and Toxicology, University of Veterinary Medicine Vienna, (5) Department of Clinical Pathology, Medical University of Vienna, (6) Biomodels Austria, University of Veterinary Medicine Vienna, (7) IFA-Tulln, University of Natural Resources and Applied Life Sciences Vienna, (8) Institute of Laboratory Animal Science, University of Veterinary Medicine Vienna, (9) Medical University of Vienna

barbara.maurer@lbicr.lbg.ac.at

STAT5 transcription factors are essential regulators of differentiation, survival and proliferation during hematopoiesis. Hyperactive STAT5 signaling requires enhanced tyrosine phosphorylation (pYSTAT5), which is frequently found in hematopoietic cancers. Importantly, recurrent gain-of-function STAT5 variants have been reported in Peripheral T Cell Lymphoma (PTCL) patients, who suffer from aggressive diseases with no targeted therapies.We aim to analyze STAT5-dosage-dependent effects on hematopoiesis. Here, we investigated whether constitutive activation of STAT5 suffices to drive PTCL and whether inhibition of the JAK/STAT pathway offers a novel therapeutic opportunity. We used cS5[upF], a hyperactive mutant of STAT5A (S710F), to generate mouse models expressing the transgene under the vav-promoter at low (vcS5[uplo]) or high (vcS5[uphi]) levels from hematopoietic stem cells on. Phenotypes were characterized by blood sampling, flow cytometric analysis and immunohistochemical stainings on organ sections. RNA-seq was done to confirm the PTCL-like disease of vcS5[uphi] mice. Effects on viability and pYSTAT5 levels of murine and human PTCL cell lines after JAK-STAT5 signalling inhibitor treatment were determined. High pYSTAT5 levels in vcS5[uphi] mice led to an aggressive expansion of CD8[up+] T cells being lethal between 25 and 45 weeks of age. In contrast, vcS5[uplo] mice developed only a mild expansion of CD8[up+] T cells with no effect on life expectancy. The vcS5[uphi] PTCL-like disease was associated with lymphadenopathy, splenomegaly and T cell infiltrations into various organs. The expression

profile determined by RNA-seq correlated closely with human PTCL forms. Treatment with a novel STAT5 SH2-domain inhibitor decreased murine and human T cell lymphoma cell lines viability in response to pYSTAT5 decline. Our results support the concept that enhanced STAT5 signaling drives PTCL and that STAT5 represents a target in these life-threatening malignancies.

111 Fasciola hepatica; identification and characterization of suspected candidates involved in Triclabendazole resistance.

Hamali, B.(1,2), Berenyi M.(2), Burger M.(1), Lipp J.(1), Kudlacek O.(1), Wischnitzki E.(2), Sehr, E.(2), Fluch, S.(2)

(1) Institute of Pharmacology, Center of Physiology and Pharmacology, Medical University of Vienna, Austria, (2)Health & Environment Department, AIT Austrian Institute of Technology GmbH, Tulln, Austria

bulut.hamali.fl@ait.ac.at

Fasciola hepatica (liver fluke) is a parasitic flatworm causing one of the most important diseases affecting animal health all over the world the so-called liver fluke disease (fascioliasis). For treatment of fascioliasis, the anthelmintic drug Triclabendazole (TCBZ) is the drug of choice for more than 25 years because of its high efficacy, both against adult and juvenile flukes. However, during the last decades an increasing number of flukes resistant to this drug have been found. We used RNA from adult flukes obtained from Northern Ireland and Lower Austria to perform next-generation sequencing of the transcriptome of the flukes. Thereby we hope to determine mutations that might confer drug resistance. Since TCBZ is a toxic substance acquired exogenously from the surrounding environment, we focused on the three phases of the detoxification pathway: Phase I (the activation phase, e.g. mainly cytochrome P450 enzymes), Phase II (the conjugation phase, e.g. Glutathione S-transferases) and Phase III (the efflux phase, e.g. mainly ABC transporters). Additionally, micro tubulin proteins known to be the target of TCBZ were included in the studies.We compared the transcripts of those sequence, between resistant and susceptible flukes on both, nucleotide and protein level. Although we could identify a new GST protein, up to now we could not find any significant change in any of the investigated proteins which can be linked to resistance phenotype.

112 Characterization of Mephedrone and its Bioactive Metabolites

Mayer, F.P. (1) Wimmer, L. (2), Partilla, J.S. (3), Burchardt, N. (1), Mihovilovic, M.D. (2), Baumann, M.H. (3), Sitte,H.H.(1 and 4)

(1)Medical University of Vienna, Center for Physiology and Pharmacology, Institute of Pharmacology, Vienna, Austria, Institute of Applied Synthetic Chemistry, Vienna University of Technology, Vienna, Austria (2),Designer Drug Research Unit (DDRU), Intramural Research Program (IRP), NIDA, NIH, Baltimore, MD, USA (3), Center for Addiction Research and Science - AddRess, Medical University Vienna, Waehringerstrasse 13A, 1090 Vienna, Austria (4)

felix.mayer@meduniwien.ac.at

The psychostimulant 4-methyl-N-methylcathinone (mephedrone, MEPH) is a major representative of former legal highs, which were sold as “bath salts”. Despite the ban of MEPH in 2011, the drug is still widely abused for recreational purposes and as doping agent. MEPH manipulates monoaminergic neurotransmission in an amphetamine-like fashion. Therefore, MEPH acts as a substrate of the high-affinity monoamine transporters (MATs) for dopamine (DAT), serotonin (SERT) and norepinephrine (NET) and triggers transporter-mediated release of neurotransmitters.Interestingly, drug abuser reports state that the action of MEPH resembles the action of 3,4-methylenedioxymethamphetamine (“ecstasy”, MDMA). However, MEPH acts as non-selective substrate at MATs whereas MDMA clearly favors SERT over DAT and NET.We sought to test the phase-1 metabolites of MEPH for their activities at MATs; as the metabolites may be responsible for the observed impact on serotonergic neurotransmission.Radiotracer-flux experiments were applied to determine the interactions between MATs and the tested metabolites in vitro and ex vivo. Single-cell FRET measurements were performed to monitor test-drug induced conformational changes in MATs in real-time. Moreover, we assessed locomotion and stereotypy in vivo in rats and determined the extracellular concentrations of dopamine and serotonin by microdialysis in nucleus accumbens.The phase 1 metabolites were found to inhibit uptake mediated by MATs with appreciable affinities in the low micromolar range and induced efflux in a dose-dependent manner. Real-time FRET measurements revealed an amphetamine-like mode of action at MATs. Consistent with in vitro findings, the test drugs mimicked the parental drug in vivo with pronounced effects on extracellular serotonin.

113 The role of microRNAs in learned safety

Ronovsky, M. (1), Böhm, V. (2), Haubensak, W. (2), Pollak, DD. (1)

(1) IMP - Research Institute of Molecular Pathology, Vienna Biocenter (VBC), Vienna, Austria, (2) Department of Neurophysiology and Neuropharmacology , Center for Pharmacology and Physiology, Medical University of Vienna , Vienna , Austria

m.ronovsky@gmail.com

114 STAT5 regulates lipid mobilisation in white adipose tissue

Kaltenecker, D. (1),Mueller, K.M. (1) (2), Moriggl, R. (1) (2) (3)

(1) Ludwig Boltzmann Institute for Cancer Research, Vienna, (2) University of Veterinary Medicine, Institute of Animal Breeding and Genetics, Vienna, (3) Medical University of Vienna, Vienna

doris.kaltenecker@lbicr.lbg.ac.at

115 Assessing basophil activation pathways via flow cytometry in the context of food allergy

Ponce, M., Diesner, S.C.(1), Moñino-Romero, S.(1), Schmidthaler, K.(1), Stark, P. (3), Hoffmann-Sommergruber, K.(4), Breiteneder, H.(4), Szépfalusi, Z.(1), and Eiwegger, T.(2)

(1) Department of Pediatrics and Adolescence Medicine, Children's Hospital, Medical University of Vienna, (2) Division of Immunology and Allergy, Food allergy and Anaphylaxis Program, The Department of Paediatrics, Hospital for Sick Children, The University of Toronto, Toronto, Canada, (3) Department of Medicine 1, Laboratory of Infection Biology, Medical University of Vienna, (4) Department of Pathophysiology and Allergy Research, Medical University of Vienna, Vienna, Austria

marta.poncecallarisa@meduniwien.ac.at

Background: Food allergy occurs at a prevalence of up to 5% in children within the first years of life. Some food allergies are associated with a high likelihood of tolerance development up to the age of five whereas others are not. The reasons are not fully understood. Currently, markers used to confirm food allergy such as specific IgE levels are not appropriate to monitor tolerance development or define sensitized non-allergic individuals. Basophil Activation Test using CD63 as a readout parameter has been described to be superior in assessing tolerance in peanut sensitized, tolerant individuals as compared to other tests. Method: In order to assess optimal kinetics of basophil activation pathways in vitro via flow cytometry in addition to CD63 measurement, children´s basophils were evaluated for CD63 and CD203c surface expression as well as for phosphorylation of ERK1/2 and p38 MAPK, ALK and PLCγ1 upon FcεRI crosslinking using flow cytometry. Results: Kinetics of phosphorylation pathway demonstrate 1 minute (ERK1/2), 3 minutes (p38) and 5 minutes (ALK) as optimal time points to measure IgE-related basophil activation. Conclusions: To understand the effect of the phosphorylation of these key intracellular proteins that contribute to basophil activation and therefore elicit allergic

symptoms is of great interest and may allow a precise delineation of events taking place during desensitization and tolerance development.

116 Studies about the antiviral innate immune response in Langerhans cells and keratinocytes

Tajpara, P(1), Kienzl, P.(1), Schuster, C.(1), Elbe-Bürger, A.(1),, Gschwandtner, M.(2), Mildner, M.(2)

(1) Medical University of Vienna, Research Division of Biology and Pathobiology of the Skin, Department of Dermatology, Vienna, Austria, (2) Medical University of Vienna, Division of Immunology, Allergy and Infectious Diseases, Department of Dermatology, Vienna, Austria

poojabahen.tajpara@meduniwien.ac.at

Introduction & Objectives: Viral infection in the skin is detected by Langerhans cells (LCs) and keratinocytes (KCs). To better understand how LCs respond functionally to viral antigens at the site of infection in the epidermis, we investigated whether pattern recognition receptors (PRRs; TLR3, MDA5, PKR, RIG-1) that recognize double stranded RNA and downstream signaling pathways can be regulated/activated in LCs upon incubation with poly(I:C) using a human ex vivo skin culture model.Material & Methods: To efficiently disrupt the physical epidermal barrier which mainly consists of the stratum corneum, normal human skin obtained from plastic surgery, was stripped sequentially 50 times. Punch biopsies were then placed in 24 well culture plates and PBS (control) or poly(I:C), a potent inducer of a strong inflammatory response in several cell types, was epicutaneously applied. Samples were harvested after 24 and 48 hours of incubation. Cryosections and epidermal sheets were prepared and analyzed for PRR expression in skin cells using immunofluorescence. Activation of NFκB (p65) and IRF3 was examined by monitoring their translocation from the cytoplasm to the nucleus in primary KCs by confocal microscopy at a single cell level.Results: We found that poly(I:C) upregulated TLR3 but not PKR in KCs and failed to upregulate/induce TLR3 and PKR in LCs. In the presence of poly(I:C), MDA5 was strongly upregulated in LCs but remained negative in KCs compared to controls. Nuclear translocation of IRF3 and p65 upon poly(I:C) treatment was observed at 2 hours and at 4 hours in KCs, respectively. Studies with LCs are ongoing.Conclusions: Our data suggest that MDA5 but not TLR3 may play a key role in the innate immune response of LCs to viral infection. Understanding the signaling events of LCs to viruses might promote development of attractive therapeutic strategies.

117 Characterization of NET response to adjuvants used in allergy vaccines

Reithofer Manuel, Dominika Polak, Claudia Kitzmüller, Barbara Bohle, Beatrice Jahn-Schmid

Department of Pathophysiology and Allergy Research, Medical University of Vienna, Vienna, Austria

manuel.reithofer@meduniwien.ac.at

Introduction: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 in humans 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 characterize the NET response of human neutrophils to alum and MPL and their possible role in the immune response induced by allergen-specific immunotherapy.Methods: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. Time course experiments to assess the amount of extracellular DNA are performed and elastase activity in supernatants determined.Results:The response to MPL showed expected similarity to the LPS-triggered NET-formation with DNA-fibers, granular myeloperoxidase, elastase or LL-37 sticking to them and intact nuclei. In contrast, alum induced cloud-like NETs, also associated with vital nuclei and granular proteins. None of the adjuvants caused cell death after 3 hours, as it was observed with PMA. In time course experiments increased amounts of extracellular DNA was observed with alum. In supernatants increased extracellular elastase activity upon stimulation with both adjuvants was observed. Conclusion:The two adjuvants induce different distributions of extracellular DNA, which shows the typical features of NETs, DNA associated with granular proteins. In order to further investigate the stimulatory capacities of these NETs, co-cultivation experiments with APCs will be performed.

118 Assessment of the impact of perinatal application of the probiotic E. coli strain O83 on the development of allergic airway inflammation in mice

Christian Zwicker (1) Mirjana Drinic (1), Priya Sarate (1), Miloslav Kverka (2), Ursula Wiedermann (1), Irma Schabussova (1)

(1) Institute of Specific Prophylaxis and Tropical Medicine, Center for Pathophysiology, Immunology and Infectiology, Medical University of Vienna, Vienna, Austria, (2) Institute of Microbiology of the Czech Academy of Sciences,v.v.i.,14220 Prague 4, CzechRepublic

christian.zwicker@meduniwien.ac.at

Clinical and experimental studies have demonstrated that the use of probiotic bacteria in different prophylactic settings, like peri- or postnatal prevention strategies, is effective in attenuating the development of allergic diseases. It has been suggested that early exposure to probiotic bacteria modulates the neonatal immune system particularly effectively. In this respect it was shown that early postnatal colonization of infants born to allergic mothers with the probiotic E. coli strain O83 was associated with a reduced prevalence of allergies in later life. In the present study we investigated whether maternal exposure to E. coli O83 during gestation and lactation can affect the development of airway inflammation in the offspring in a mouse model of type I allergy.Surprisingly, the offspring derived from E. coli O83-treated dams showed significantly increased airway hyperresponsiveness (AHR) after exposure to ovalbumin compared to mice from PBS-treated mothers. Increased AHR in mice born to bacteria-treated mothers was not associated with enhanced systemic IgE levels or elevated numbers of eosinophils in broncheoalveolar lavage fluid. However, we detected significantly increased IL-4 levels in the lungs of mice derived from E. coli O83-treated mothers but decreased levels of the typically AHR-associated cytokines IL-5 and IL-13 compared to control mice. Moreover, increased mucus depositions and mucin mRNA levels were observed in the lungs isolated from the offspring of E.coli O83-treated mothers compared to offspring of control mothers.Here, we show that perinatal maternal supplementation with E. coli O83 leads to increased AHR as well as elevated IL-4 and mucus production in lungs of their offspring. The role of IL-4 in this phenotype and its cellular source still need to be determined. This study demonstrates that the use of E. coli O83 for the prevention of allergic diseases can have opposing effects exacerbating allergic airway inflammation in mice.

119 CD98hc: a putative novel prognostic biomarker in pancreatic ductal adenocarcinoma

Bianconi, D. Herac M., Heller G., Gleiss A.3, Heinze G.3, Unseld M. , Schindl M., Scheithauer W. , Zielinski CC. , Prager G.

Medical University of Vienna

daniela.bianconi@meduniwien.ac.at

120 Riociguat, a stimulator of the soluble guanylate cyclase, reduces liver cirrhosis and portal hypertension in rats with biliary liver cirrhosis.

Supper, P. (1) Schwabl, P. (2), Bauer, D. (3), Brusilovskaya, K. (4), Riedl, F. (5), Strobel, B. (6), Rohr-Udilova, N. (7), Hayden, H. (8), Podesser, B. (9), Peck-Radosavljevic, M. (10), Trauner, M. (11), Reiberger, T. (12)

Medical University of Vienna

n1342213@students.meduniwien.ac.at

In liver cirrhosis the nitric oxide (NO) signalling pathway including its intracellular receptor, the soluble Guanylat-Cyclase (sGC) is disturbed. Riocyguat (RIO) serves as a sGC-agonist and is approved for treatment of pulmonary Hypertension. In addition to its vasodilatory properties, antifibrotic effects have been described. Thus we investigated the effect of RIO on the portal hypertensive syndrome in rats with biliary liver cirrhosis.In order to induce liver cirrhosis, male Sprague-Dawley-Rats underwent bile duct ligation (BDL), whereas the control group received a sham operation (SO). Daily gavage of 1mg/kg RIO or placebo (VEH) were administered as therapy. In a preventive setting, rats were treated on day 7-21 post BDL, while in a therapeutic setting therapy was administered from day 21-35.Heart rate (HR), mean arterial pressure (MAP), portal pressure (PP) and blood flow of the superior mesenteric artery (SMABF) were measured at the end of treatment. Porto-systemic shunting (PSS) was determined using coloured microspheres. Liver fibrosis was quantified by chrome-aniline-blue (CAB) staining and hydroxyproline-concentration.In the prevention setting, the BDL-VEH cohort developed pronounced fibrosis, which was ameliorated by treatment in the BDL-RIO group (CAB: 23.7±4.6 vs.13.3±2.1%, $p<0.001$; HP: 286±147 vs.144±74 µg/g,$p=0.039$). Furthermore, a significant decrease in PP was detected (13.2±2.5 vs.10.1±2.4 mmHg, $p=0.048$), while MAP, HR, SMABF and PSS were not influenced by RIO.In the therapeutic setting BDL-VEH animals presented with severe liver-cirrhosis, which was reduced in BDL-RIO rats (CAB: 29.9±2.2 vs 19.3±5.7%, $p<0.001$; HP: 354±169 vs.233±45 µg/g, $p=0.044$). While MAP, HR, SMABF and PSS were similar in BDL-VEH and BDL-RIO animals, PP was significantly lower in BDL-RIO-animals (15.5±1.6 vs.11.9±2.1 mmHg, $p=0.002$).In rats with biliary cirrhosis Riociguat treatment reduces liver damage and portal pressure, without impairing systemic hemodynamics.

121 Riociguat, a stimulator of the soluble guanylate cyclase, prevents liver fibrosis cirrhosis and development of portal hypertension in a CCL4 rat model

Bauer D. (1), Schwabl P. (2), Supper P. (3), Brusilovskaya K (4), Riedl F (5), Strobel B (6), Rohr-Udilova N(7), Hayden H(8), Podesser B(9), Peck-Radosavljevic M(10), Trauner M(11), Reiberger T(12)

Division of Gastroenterology and Hepatology, Department of Internal Medicine III, Medical University of Vienna

david.bauer@meduniwien.ac.at

Introduction: In liver cirrhosis the signalling function of nitric oxide (NO) is perturbed, partially due to impaired activity of the soluble guanylyl cyclase (sGC). Riociguat (RIO) is a NO-independent stimulator of the sGC with potential anti-fibrotic effects. The aim of this study was to assess the effects of RIO in experimental toxic liver fibrosis and portal hypertension.Methods: Liver cirrhosis was induced in Sprague Dawley rats by intraperitoneal injection of Carbon tetrachloride (CCl4) for 8 weeks, while the control groups received olive oil (OO). The dosage of CCl4 in the preventive setting was 0.5ml/kg and 1ml/kg in the therapeutic setting. From weeks (W) 5-8 RIO (1mg/kg) or placebo were gavaged daily. At the end of W8 hemodynamics including heart rate (HR), mean arterial pressure (MAP), portal pressure (PP), superior mesenteric arterial blood flow (SMABF) and porto-systemic shunting (PSS) were assessed. Hepatic fibrogenesis was quantified using chrome-blue-aniline (CAB) staining and measurement of the hydroxyproline content (HP).Results: In the preventive setting, RIO treatment reduced fibrogenesis (CAB: 33.5±4.9 vs. 25.8±2.8%, p<0.028; HP: 194±48 µg/g vs. 160±42, p=n.s.) and caused a significant decrease in PP (8.1±1.3 vs. 6.4±0.4, mmHg, p=0.042) and SMABF (13.7±1.4 vs. 10.9±1.8, mL/min/kg, p=0.034), without affecting MAP and or HR. CCl4[RIO animals also tended to have lower PSS (35.4±29.8 vs. 5.8±8.7, %, p=0.111).In the therapeutic setting, CCl4 animals developed marked cirrhosis with ascites. CCl4-RIO animals presented a trend towards less fibrosis (CAB: 28.8±7.5 vs. 43.8±6.1, p=0.056), but showed no significant amelioration of hemodynamics as compared to CCl4-VEH rats.Discussion: In the hepatotoxic model of CCl4 cirrhosis, the positive anti-portal hypertensive and anti-fibrotic effects of RIO treatment are most pronounced in an early preventive setting, whereas in advanced cirrhosis stimulation of sGC exerts only minor effects.

122 Factors determining flicker-induced retinal vasodilatation in healthy subjects

Mozhgan Sharifi Zad

Medical University of Vienna

mozhgan.sharifizad@meduniwien.ac.at

Purpose: To analyze factors determining the retinal arterial and venous response to stimulation with diffuse luminance flicker in healthy subjects.Setting: In the recent years evidence has accumulated that flicker-induced retinal vasodilation is altered in a variety of ocular and systemic diseases. In the present study we set out to analyze determinants of flicker-induced vasodilatation in healthy subjects.Results:Flicker responses in arteries and veins were more pronounced in protocol 2 as compared to protocol 1 ($p < 0.001$ each). In both protocols the vascular response to stimulation with diffuse luminance flicker was larger in smaller vessels (p values between 0.001 and 0.016). In protocol 2 the retinal arterial flicker response was

negatively associated with cholesterol serum levels (p = 0.033), in protocol 1 only a tendency towards this effect was observed (p = 0.056).The present analysis indicates that retinal arterial and venous responses to stimulation with diffuse luminance flicker depend on the way the stimulation is delivered through the fundus camera. In addition, the flicker response varied with the vessel size, the smaller the vessel width, the larger the flicker response. Finally our data indicate that even within the normal range higher cholesterol serum levels are associated with lower hyperemic flicker responses.

123 Application of a humanized OPN mouse model in immuno-metabolic studies

Grün, N.G. (1), Schuch, K. (1), Moreno-Viedma, V. (1), Strohmeier, K. (1), Zeyda, M. (2) and Stulnig, T.M. (1)

(1) Christian Doppler Laboratory for Cardio-Metabolic Immunotherapy and Clinical Division of Endocrinology and Metabolism, Department of Medicine III, Medical University of Vienna, Vienna, Austria, (2) Department of Pediatrics and Adolescent Medicine, Clinical Division of Pediatric Pulmonology, Allergology and Endocrinology, Medical University of Vienna, Vienna Austria

nicole.sommer@meduniwien.ac.at

The multifunctional protein osteopontin (OPN) is involved in a number of inflammatory processes, including obesity-linked complications, asthma, rheumatoid arthritis and cancer. Proteolytic cleavage of OPN via thrombin and matrix metalloproteinases increases OPN's pro-inflammatory properties and therefore constitutes an interesting target to develop new strategies to counteract OPN's actions. Here we created a humanized SPP1 (hSPP1) mouse model to on the one hand enable research on targeting human OPN, e.g. by active immunization, and on the other hand to evaluate its suitability as a model for obesity-triggered adipose tissue inflammation and insulin resistance. Male hSPP1 and C57BL/6JRj wild-type (WT) mice were fed a high-fat (HF) or low-fat (LF) diet for 22 weeks, respectively. Body weight, insulin resistance, adipose tissue and liver inflammation markers as well as adipocyte hypertrophy were determined. Importantly, untreated hSPP1 animals did not show any difference in body weight, glucose levels and behavioral biology in comparison to WT mice. Moreover, hSPP1 mice similarly developed obesity-associated insulin resistance and tissue-specific inflammation. Interestingly, transgenic modification affected OPN expression patterns in hSPP1 animals compared to WT mice.Therefore we propose that hSPP1 mice can be used to target human OPN in obesity- and cardio-metabolic diseases and probably a wide variety of other inflammatory or malignant disorders. 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.).

124 Identification of matrix metalloproteinase-12 as a candidate molecule for prevention and treatment of cardiometabolic disease

Amor, M (1), V Moreno-Viedma (1), A Sarabi (1), NG Grün (1), B Itariu (1), L Leitner (1), I Steiner (2), M Bilban (3), K Kodama (4), A J Butte (4), G Staffler (5), M Zeyda (6), TM Stulnig (1)

(1) Christian Doppler Laboratory for Cardio-Metabolic Immunotherapy and Clinical Division of Endocrinology and Metabolism, Department of Medicine III, Medical University of Vienna, Vienna, Austria, (2) Center for Medical Statistics, Informatics, and Intelligent Systems, Section for Medical Statistics, Medical University of Vienna, Austria. (3) Core Facility Genomics, Core Facilities, Medical University of Vienna, Vienna, Austria, (4) Institute for Computational Health Sciences. University of California, San Francisco, EEUU. (5) AFFiRiS GmbH, Vienna, Austria. (6) Department of Pediatrics and Adolescent Medicine, Clinical Division of Pediatric Pulmonology, Allergology and Endocrinology, Medical University of Vienna.

melina.amor@meduniwien.ac.at

Obesity is strongly associated with the metabolic syndrome, a compilation of factors conferring considerable risk for cardiometabolic disease, i.e. cardiovascular disease and type 2 diabetes mellitus. Reversing or controlling the worldwide epidemic of metabolic syndrome requires novel interventions to address this substantial health challenge. The objective of this study was the identification of candidate molecules for the simultaneous prevention and treatment of insulin resistance and atherosclerosis, conditions that underlie type 2 diabetes and cardiovascular disease, respectively. Therefore we used an unbiased bioinformatics approach to identify factors that are upregulated in both conditions by combining data from a microarray experiment comparing gene expression in white adipose tissue and atherosclerotic aorta of obese and atherosclerotic mice compared to lean controls with a meta-analysis of published microarrays investigating atherosclerotic vessels and a published meta-analysis on type 2 diabetes. We obtained a pool of eight genes that were dysregulated in all the considered databases. These included well-known as well as novel potential targets for treatment of type 2 diabetes and cardiovascular disease. Matrix metalloproteinase 12 [MMP12] was chosen for further validation, which was done at mRNA, protein and activity levels, in visceral and subcutaneous white adipose tissue from obese compared to lean mice and humans. The results convincingly confirmed the positive regulation of MMP12 in obesity. In conclusion, an interesting pool of potential targets for treatment and prevention of cardiometabolic disease was identified with MMP12 being one confirmed interesting molecule.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).

124A **CNS leptin signaling increases hepatic VLDL secretion and reduces hepatic *de novo* lipogenesis**

M. T. Hackl[1], C. M. Schuh[1], S. Abu Eid[1], M. Krssak[1], C. Fuernsinn[1], C. Buettner[2], T. Scherer[1]

[1] Division of Endocrinology and Metabolism, Department of Medicine III; Medical University of Vienna, Austria
[2] Departments of Medicine and Neuroscience and Diabetes, Obesity and Metabolism Institute (DOMI), Icahn School of Medicine at Mt Sinai, NY, USA
This study was supported by the Austrian Science Foundation FWF Grant #26766 to T.S

martina.hackl@meduniwien.ac.at

Non-alcoholic fatty liver disease (NAFLD) is characterized as hepatic steatosis caused through an imbalance between uptake and production versus export and utilization of triglycerides (TG) in the liver, resulting in hepatic lipid accumulation. The adipokine leptin is involved in the regulation of hepatic TG content. Leptin deficient lipodystrophic patients suffer from sever NAFLD, while leptin treatment dramatically ameliorates steatosis in these patients. Leptin may reduce steatosis by boosting hepatic TG export, however, the exact mechanisms remain unclear. Since leptin exerts its function mainly via signaling in the central nervous system (CNS), we hypothesized that brain leptin modulates hepatic TG secretion. To study the role of CNS leptin on hepatic TG flux isolated brain hyperleptinemia was achieved by infusing leptin or vehicle directly into the 3rd ventricle of male Sprague Dawley rats via stereotaxic intracerebroventricular (ICV) cannulae during acute (4 hour) tyloxapol experiments. Rats were infused with leptin, a leptin receptor antagonist or vehicle over 4 weeks using osmotic mini-pumps attached to stereotaxic cannulae. Hepatic lipid content was assessed non-invasively using 1H-MRS. Acute leptin ICV infusions markedly increased hepatic TG export which was in agreement with chronic leptin infusions that reduced hepatic fat content. These changes where mirrored by a reduction in multiple hepatic lipid species and a suppression in key de novo lipogenic proteins assessed by immunoblots of liver tissue samples. Conversely, blocking endogenous CNS leptin signaling chronically by ICV infusion of the antagonist resulted in hepatic steatosis. In HFD-challenged rats the effects of brain leptin on hepatic steatosis were absent, suggesting that obesity alters the ability of CNS leptin to promote TG secretion. These studies identify CNS leptin signaling as an important regulatory factor in determining hepatic lipid content.

124B **The impact of obesity on the humoral and cellular immune response to tick-borne-encephalitis (TBE) vaccination**

Stefan M. Schulz[1], Eva Maria Pöllabauer[1], Ines Zwazl[1], Claudia Seidl-Friedrich[1], Dejana Dijanovic[1], Angelika Wagner[1], Erika Garner-Spitzer[1], Ursula Wiedermann[1]

[1]Institute of Specific Prophylaxis and Tropical Medicine, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna

stefan.schulz@meduniwien.ac.at

Obesity has been associated with immune impairment and evidence suggests that it also affects vaccine efficacy in this growing patient group.

The aim of this single center, open label, phase IV clinical trial is to assess the impact of obesity on vaccinations and to gain a more detailed insight into the deviations of the cellular and humoral responses to the vaccine.

In the current study 37 adult, obese (BMI>30) and 37 control (BMI<25; age and gender matched) patients receive a TBE booster vaccine and blood is drawn before and 7 days, 1 and 6 months post vaccination. For immunological analysis vaccine specific antibody levels (TBE-NT), immunoglobulin levels and cytokine production (IL-2, IFN[gamma], IL-10, TNF[alpha], IL-6) in PBMCs upon TBE stimulation are evaluated. Characteristics of lymphocyte populations in obese versus healthy weight vaccinees are analysed and compared. Furthermore the influence of metabolic and hormonal factors on vaccine responses are evaluated prior to vaccination (by measuring testosterone, estrogen, progesterone, FSH, LH, cholesterol, triglycerides, HDL, LDL, (apo-)lipoproteins, glucose, fructosamin, insulin, CRP, as well as leptin and its receptor).

Currently 13 adipose and 13 matched control vaccinees have finished the study. Evaluation of metabolic and hormone parameters revealed significantly higher levels of insulin, triglycerides, CRP, LDL, Lp(a) and ApoB in the obese group and significantly higher levels of fructosamin, HDL and ApoA1 in the control group. No differences in hormone parameters were observed. Analysis of the reactogenicity profile showed a trend towards increased intensity and duration of systemic reactions to vaccination in the obese group. The antibody measurements as well as the cellular parameters are currently under investigation.

This study will give insight into the immunological properties and possible impairments of vaccine responses in obesity and may lead to changes of vaccination schedules and procedures.

124C Psychosomatic patients in integrated care - Which treatment mediators do we have to focus?

Parth, K. [1], Rosar, A. [2], Stastka, K. [3], Zimmermann, T. [4], Storck, T. [5], Löffler-Stastka, H. [6]

[1] Medical University Vienna

[2] OWS Psychiatrische Abteilung, [3] KFJ Psychiatrische Abteilung/SMZ Süd, [4] Psychologische Hochschule Berlin, [5] Psychologische Hochschule Berlin, [6] Medizinische Universität Wien

karoline.parth@meduniwien.ac.at

125 A novel Dopamine circuit for writing Emotional Memory

Groessl, F. (1) Munsch, T. (2), Meis S. (2), Griessner J. (1,4), Pliota P. (1), Badurek S. (2), Haubensak W. (1,3), Lessmann V. (

IMP Vienna, Otto-von-Guerike Universität Magdeburg, CSF Vienna, Medical University of Vienna

Florian.groessl@imp.ac.at

126 High stability of BOLD response for emotional faces at 7 Tesla

Geissberger, N. (1) Sladky, R. (1), Tik, M. (1), Hoffmann, A. (1), Woletz, M. (1), Willinger, D. (1), Windischberger, C. (1)

Medical University of Vienna

nicole.geissberger@meduniwien.ac.at

127 Selective muscular inflammation in an animal model of neuromyelitis optica

Kaufmann, N. (1)

Medical University of Vienna

nathalie.kaufmann@meduniwien.ac.at

Neuromyelitis optica (NMO-SD) is an autoimmune disease of the CNS characterized by astrocyte-destructive lesions in spinal cords and optic nerves. In a majority of patients it is characterized by the presence of NMO-IgG, autoantibodies directed against the water channel aquaporin 4 (AQP4). Although AQP4 is expressed in other organs, e.g. fast twitch skeletal muscles and kidney collecting duct epithelial cells, they are essentially spared from destruction. However, our lab has described skeletal muscle inflammation in our NMO-SD animal model in the past, and this was later corroborated by a human case report. Here we report that in our experimental animals highly encephalitogenic AQP4-specific T cells together with NMO-IgG initiate not only inflammation of some skeletal muscles but also caused pronounced

inflammation of the extraocular muscles. We observed infiltration of macrophages and inflammatory cells as well as complement deposition on the sarcolemma, indicating that the extraocular muscles are destroyed by antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cellular cytotoxicity (CDCC). In contrast, predominantly connective tissue components were invaded in skeletal muscles, while the muscle fibres were spared. The same observations were made when rats were injected with myelin basic protein (MBP)-specific T cells. While this might seem surprising, MBP is available from myelinated peripheral motor nerve fibres in the muscle tissue. In contrast, myelin oligodendrocyte glycoprotein (MOG), which is found at 10-fold lower mRNA levels in the PNS than MBP, could not induce muscular inflammation. These observations indicate that T cells and antibodies do not have to recognize the same antigen in order to cause destruction of muscle fibres, but that the target antigens of antibodies and T cells must be present in the affected tissue. It also indicates that extraocular muscles are better targets for ADCC and CDCC than skeletal muscles.

128 Microarray analysis of RNA isolated from formalin fixed, paraffin embedded and fresh frozen brain tissue

Tröscher, AR.(1), Wimmer, I.(2), Brunner, F.(3), Bauer, J.(4)

Center for Brain Research, Vienna

anna.troescher@meduniwien.ac.at

A considerable number of epilepsy patients do not improve with anticonvulsive medication. Most undergo epileptic surgery to remove the affected brain region which is subsequently formalin fixed and paraffin embedded (FFPE) for histopathological analysis. FFPE tissue can be stored over decades. Therefore, large repositories are available. Being able to use these tissues for gene expression analysis would be beneficial for identifying underlying processes of the disease. Therefore we optimized RNA isolation from FFPE tissue and compared the achieved data with matched fresh-frozen (FF) tissues. Three epileptic surgery samples from Rasmussen encephalitis patients were used for this study. One half of the sample was immediately snap frozen and the other half paraformaldehyde fixed and embedded in paraffin. RNA was isolated using various RNA isolation kits. RNA quality and yield was determined using the Agilent bioanalyzer. RNA quality was additionally checked with qRT-PCR for standard housekeeping genes. After satisfactory RNA quality and yield were achieved whole genome Affymetrix microarrays were performed.RNA isolation from small amounts of FFPE tissue was feasible. Due to protocol optimization the yield could be highly increased and the quality was satisfactory. To determine whether it is possible to analyze the extracted RNA on a gene expression level, qRT-PCR was performed. Expectedly, the yield of RNA from the FF tissue was higher. However, also from the FFPE material we were able to detect various house keeping genes. The whole genome microarray analysis led to similar results. From 48.226 genes 42.902 genes were

not significantly differently expressed between FF and FFPE tissue. Therefore FFPE tissues can be used as a valuable source for gene expression analysis.With this method the big archive of FFPE samples can be unlocked and gene expression profiles of many diseases can be characterized. This especially is important for investigation of rare diseases.

129 Establishment of a new adhesion model at the sciatic nerve in the rat

Lemke, A. (1,2), Penzenstadler, C (1), Lidinsky, D (1,2), Hopf, R (1,2), Nürnberger, S (1,3), Redl, H (1,2), Bradl, M (4), Wolbank, S (1,2), Schmidhammer, R (1,2)

(1) Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Vienna, Austria, (2) Austrian Cluster for Tissue Regeneration, (3) Bernhard Gottlieb University Clinic of Dentistry, Vienna, Austria; (4) Department for Neuroimmunology, Center for Brain Research, Medical University of Vienna, Austria

angela.lemke@trauma.lbg.ac.at

Adhesions, scarring and fibrosis due to repair or reoperation of peripheral nerves represent major challenges in health care. Up to now no adequate medical treatment exists to prevent post-surgical scarring and therefore operative adhesiolysis, such as external neurolysis, is required. However, adhesions recur in most patients and every surgical intervention causes new scarring leading to progressive damage. In this study we aimed to establish a new rat model, which mimics the formation of post-operative adhesions at the sciatic nerve by applying fibrosis-inducing glue, containing glutaraldehyde. This application should reproducibly induce a locally restricted site of extensive scarring and adhesion. This would, in a next step, enable the investigation of different anti-adhesive components. Adhesion formation was induced by applying fibrosis-inducing glue after exposure of the sciatic nerve in female sprague dawley rats. At different time points scarring was analyzed via macroscopical and microscopical evaluation. Moreover, functional characterization was conducted by CatWalk© gait analysis and electrophysiological analysis. After 3 and 4 weeks, strong adhesions were clearly visible macroscopically after re-exposure of the sciatic nerve. Microscopic evaluation of tissue sections showed the formation of internal fibrosis and collagen fibers surrounding the nerves compared to controls. Functional examination via gait analysis showed a clear reduction of (intermediate) toe spread after glue application. Also, electrophysiological analysis indicated an impairment of the nerve functionality compared to controls. We established a new adhesion model, which reproducibly induces the formation of strong fibrous strands around the nerve and internal fibrosis, leading to reduced nerve functionality. As a next step, anti-adhesive and anti-fibrotic components can be evaluated in order to find new therapeutics against nerve scarring.

130 Formation of GABA[downA] receptor complexes containing [alpha]1 and [alpha]5 subunits is paralleling a multiple T-maze learning task in mice

Szodorai, E.§ (1,2), Ghafari, M.§ (1), Falsafi, SK.§ (1), Kim, EJ. (1), Li, L. (1), Höger, H. (3), Berger, J. (4), Fuchs, K. (2), Sieghart, W. (2), Lubec, G. (5)

(1) Department of Pediatrics, Medical University of Vienna, (2) Department of Molecular Neurosciences, Center for Brain Research, Medical University of Vienna, (3) Core Unit of Biomedical Research, Division of Laboratory Animal Science and Genetics, Medical University of Vienna, (4) Department of Pathobiology of the Nervous System, Center for Brain Research, Medical University of Vienna (5) Department of Pharmaceutical Chemistry, University of Vienna

edit.szodorai@meduniwien.ac.at

There is limited information on the role of GABA type A receptors (GABA[downA]Rs) containing [alpha]1, [alpha]5 and [gamma]2 subunits in learning and memory. Here, we assessed the possible role of such receptors in spatial learning using the multiple T-maze (MTM) paradigm.C57BL/6J mice were trained in the MTM which induced elevated levels of [alpha]1 and [alpha]5 subunit-containing hippocampal GABA[downA]R complexes. Moreover, spatial learning evoked a significant increase in the colocalization of [alpha]1 and [alpha]5 subunits in both, CA1 and dentate gyrus regions of the hippocampus suggesting the formation of complexes containing both subunits. Additionally, the presence of [alpha]1, [alpha]5 and [gamma]2 subunits in high molecular weight GABA[downA]Rs was detected and significant correlation in the level of [alpha]1-containing complexes with those containing [alpha]5 and [gamma]2 subunits was demonstrated. Accordingly, [alpha]1 deficiency led to decreased level of [gamma]2 subunit-containing complexes, however, had no effect on [alpha]5-containing ones. On the other hand, [alpha]1 knock out (KO) mice showed impaired performance in the MTM correlating with increased level of [alpha]5 subunit-containing GABA[downA]Rs in comparison to trained floxed control animals which quickly learned the task. Taken together, these results suggest that [alpha]1, [alpha]5 and [gamma]2-containing hippocampal GABA[downA]R complexes play an essential role in spatial learning and memory in which targeted disruption of the α1 subunit produces profound deficits. §equally shared authorship.

131 Characterizing endocytosis of muscle specific kinase upon ligand activation and its role in the formation of the neuromuscular junction

Geid, L. (1), Lipp, A. (2), Parzer, S. (3), Herbst, R. (4)

Medical University of Vienna

laura.geid@meduniwien.ac.at

The neuromuscular junction [NMJ] connects a motor neuron and a muscle fiber. The muscle specific kinase [MuSK] is the key signaling molecule at the NMJ, it is required for the formation of a mature and functional NMJ. In mice lacking MuSK, motor neurons will overgrow the muscle fiber and no postsynaptic specializations, such as the clustering of acetylcholine receptor [AChR], are found. MuSK is a receptor tyrosine kinase [RTK] and its activation requires agrin, a large extracellular matrix protein, secreted by the motor neurons at the NMJ, as well as the co-receptor Lrp4. In general, RTKs are internalized rapidly upon ligand binding and routed intracellularly either for degradation or recycling. In addition, signaling from endosomes can activate specific pathways. Therefore, these processes can influence signaling of RTKs. The aim of the project is to characterize agrin-dependent MuSK endocytosis and the role in NMJ formation and maintenance. We developed muscle cell systems to study MuSK endocytosis. To determine the influence of MuSK endocytosis on subsequent signaling, pharmacological inhibition as well as inducible RNAi-mediated knockdown of specific endocytic proteins are the methods of choice. To assess the impact of those proteins on MuSK endocytosis upon agrin treatment, live imaging in muscle cells in order to follow MuSK internalization will be performed. To complement the data from live cell microscopy, the amount of surface MuSK upon different treatments will be determined by biochemical analyses. As a readout for MuSK signaling upon agrin treatment and inhibition of endocytosis clustering of AchRs will be analyzed. In this way, we expect to elucidate how MuSK signaling is influenced by endocytosis.

132 Maternal immune activation epigenetically regulates hippocampal serotonin transporter levels

Reisinger, S. N., Kong, E., Cabatic, M., Khan, D., Schulz, S., Pollak, D.D.

Medical University of Vienna

sonali.reisinger@meduniwien.ac.at

Major depressive disorder (MDD) is one of the most debilitating psychiatric diseases, affecting a large percentage of the population worldwide. Currently, the underlying pathomechanisms remain incompletely understood, hampering the development of critically needed alternative therapeutic strategies, which further largely depends on the availability of suitable models systems.Here we used a mouse model of early life stress – a precipitating factor for the development of MDD – featuring infectious stress through maternal immune activation (MIA) by polyinosinic:polycytidilic acid (Poly(I:C)) to examine epigenetic modulations as potential molecular correlates of the alterations in brain structure, function and behavior induced by MIA. We found that in adult female MIA offspring anhedonic behavior was associated with modulations of the global histone acetylation profile in the hippocampus and specific changes at

the promoter and expression of the serotonin transporter (SERT), critically involved in the etiology of MDD and pharmacological antidepressant treatment. Furthermore, an accompanying reduction in hippocampal levels of histone deacetylase (HDAC) 1 was observed in MIA as compared to control offspring.Based on these results we propose a model in which the long-lasting impact of MIA on depression-like behavior and associated molecular and cellular aberrations in the offspring is brought about by the modulation of epigenetic processes and consequent enduring changes in gene expression. These data provide additional insights into the principles underlying the impact of early infectious stress on the development of MDD and may contribute to the development of new targets for antidepressant therapy.

133 Development and characterization of a human Rett syndrome cell model using a transient non-integrating reprogramming strategy

Ackerbauer, D., Steinkellner, H., Laccone, F.

Medical University of Vienna, Institute for Medical Genetics

daniela.ackerbauer@meduniwien.ac.at

INTRODUCTION: Classical Rett Syndrome (RTT) is caused by mutations in the MeCP2 gene, a transcriptional modulator, highly abundant in the mammalian brain and crucial for CNS development. The purpose of this study is to establish a human cell model for RTT via a non-integrating direct reprogramming method, to accurately characterize it and to evaluate the outcome of an in vitro protein replacement therapy. METHODS: Fibroblasts of a male RTT patient and from a healthy control were transfected with episomal plasmids coding for Pax6 and Sox2 transcription factors. Cells were kept under specific reprogramming culture conditions for 30 days post transfection followed by a weekly replating protocol for additional 3 weeks. Morphological changes and expression of neural progenitor specific genes were monitored during this reprogramming process. The induced neural progenitor cells (iNPs) were then used for neural and astrocytic differentiation. Differentiated cells were stained for neuron-specific markers Tuj1, MAP2 and Syn1 as well as for the astrocytic marker S100b in IF.RESULTS: Morphological changes were observed in bright field microscopy throughout the reprogramming process and RT-qPCR analysis revealed elevated mRNA expression levels of the neural markers HOXB9, NCAM1 and Nestin together with the progenitor cell-associated genes SOX2, PAX6, FOXG1 and OCT3/4. The iNPs were then differentiated into neuronal cells, showing typical neuronal morphology and expressing Tuj1 and MAP2 neuronal markers as well as the synaptic protein Syn1.CONCLUSION: We demonstrated the successful reprogramming of fibroblasts of a male RTT patient as well as from a healthy control into iNPs using a transient non-viral transfection strategy. These iNPs were able to differentiate into the neural lineage expressing defined neural and synaptic markers. To further characterize our in vitro RTT cell model, electrophysiological and RNA-Seq analyses are planned.

134 Human amniotic membrane - a promising producer of pulmonary surfactant

Lemke, A. (1,2), Klotz, A (1), Lindenmair, A (1,2), Nürnberger, S (1,3), Hennerbichler, S (2,4), Redl, H (1,2), Wolbank, S (1,2)

(1) Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Vienna, Austria, (2), Austrian Cluster for Tissue Regeneration, (3) Bernhard Gottlieb University Clinic of Dentistry, Vienna, Austria; (4) Red Cross Blood Transfusion Service Upper Austria, Linz, Austria

angela.lemke@trauma.lbg.ac.at

135 2-linoleoyglycerol is an ancestral endocannabinoid at CB1 cannabinoid receptors in signaling networks reconstructed in Drosophila melanogaster

W. Johannes Beiersdorf(1), Giuseppe Tortoriello(2), Gareth Williams(3), Gary A. Cameron(4), Heather B. Bradshaw(5), Ken Mackie(5), Michael Williams(6), Vincenzo Di Marzo(7), Leanne LU(3), Patrick Doherty(3), Thomas Hummel(8), Tibor Harkany(1)

(1) Center for Brain Research- Medical University of Vienna, Department of Molecular Neurosciences, Vienna, Austria. (2) Karolinska institutet, Molecular Neurobiology, Stockholm, Sweden. (3) King's College London, Wolfson Centre for Age-related Diseases, London, United Kingdom. (4) University of Aberdeen, schools of Applied Medicine and Dentistry, Aberdeen, United Kingdom. (5) Gill Center for Biomolecuolar Science, Department of Psychological and Brain Sciences, Indiana- Bloomington, USA. (6) Uppsala University, Department of Neuroscience, Uppsala, Sweden. (7) Istituto di Chimica Biomoleculare, Endocannabinoid Research Group, Naples, Italy, (8) Department for Neurobiology, University of Vienna, Vienna, Austria

johannes.beiersdorf@meduniwien.ac.at

136 TTV load in kidney transplanted children

Heilos, A. (1), Uhl, P. (2), Arbeiter, K. (3), Aufricht, C. (4), Müller-Sacherer, T. (5), Heindl-Rusai, K. (6)

Medical University Vienna, Austria, Department of Paediatrics and Adolescent Medicine, Division of Paediatric Nephrology and Gastroenterology

andreas.heilos@meduniwien.ac.at

Chronic deterioration of graft function is mainly caused by antibody-mediated rejection, medication side effects and infectious complications all linked to either under- or over-immunosuppression (IS). In the paediatric population, the individual status of IS is of high relevance since (I) children are at higher risk of over-IS than adults and (II) adolescents are more prone to under-IS because of non-adherence. Today, the IS is monitored by measurement of calcineurin inhibitor or mTOR inhibitor blood levels.Assessment of Torque teno virus (TTV) viral load could be an alternative and easy method to assess the quality of IS in transplanted patients. TTV is a non-enveloped, single-stranded, circular DNA virus belonging to the family anelloviridae. It is present in the plasma of >90% of individuals and can even be detected in 90% of children by the age of 3.5 years. Since TTV has no known disease inducing capacity, detectable changes do not have direct therapeutic consequences and could, therefore serve as a diagnostic and prognostic marker in transplanted patients.Data from adult studies prove that TTV load increases shortly after lung transplantation and is significantly influenced by the IS regime in liver transplanted adults and children.Experimental procedure: The first aim of this study was to determine the TTV load in kidney transplanted children before and after transplantation in order to detect changes due to the IS. The second objective was to cross-sectionally monitor the TTV load for a period of one year and analyse standard deviation, coefficient of variation and their association with age, gender, post-transplant time and IS-regime.In this study, 45 kidney transplanted children were involved. The next step will be to analyse correlations between TTV load and IS medication trough levels and signs of under- or over-IS caused by non-adherence or as a consequence of infectious episodes in the individual patients, respectively.

137 PDGF pathway privileged in transcriptome of injured mesothelial cells is repressed by lithium

Bialas, K. (1, 2), Rusai, K. (2), Herzog, R. (1, 2), Unterwurzacher, M. (1, 2), Wagner, A. (1, 2), Aufricht, C. (2), Kratochwill, K. (1, 2)

(1) Zytoprotec GmbH, Vienna, Austria (2) Department of Pediatrics and Adolescent Medicine, Medical University of Vienna, Vienna, Austria

katarzyna.bialas@meduniwien.ac.at

Following exposure to peritoneal dialysis fluids (PDF), mesothelial cells exhibit features of injury. The observed cell damage was described to be associated with inadequate cellular stress responses (CSR) as a result of activation of glycogen synthase kinase-3β (GSK-3β). In immortalized mesothelial cells adequate CSR could be restored by lithium, a well described GSK-3β inhibitor. The lithium dependent cytoprotection, however, might be mediated by various

mechanisms of which identification is desirable to advance clinical application of lithium as cytoprotective additive to PDF. For this purpose a transcriptome-wide study on human primary mesothelial cells (HPMC) exposed to PDF (Extraneal®, Baxter) or PDF supplemented with lithium was performed and the transcriptome was investigated with gene expression microarrays (Affymetrix). The biological pathways showing the PDF/lithium dependence were characterized using the PANTHER database. Cell injury triggered by PDF was associated with significantly differential expression of genes overrepresented within platelet derived growth factor (PDGF) pathway. Those effects were independent of GSK-3β activation. PDF supplemented with lithium caused lower cell injury and did not result in overrepresentation of the PDGF pathway. Expression of PDGFA upregulated by PDF was attenuated with addition of lithium. Expression of migratory proteins was suppressed when PDGFA siRNA was applied during exposure of HPMC to PDF. PDGFA release to effluent of PD patients correlated with the time on PD.The observed decrease of cell injury and lack of overrepresentation of PDGF pathway as well as lower expression of PDGFA confirm the cytoprotective potential of lithium in HPMC. PDGFA was identified to induce migration in-vitro and investigation of clinical material suggests its role in early CSR to PDF. The relevance of these findings in context of GSK-3β related cytoprotection will be subject to confirmation in further in-vivo studies.

138 Blockade of HLA antibody-triggered classical complement activation by a humanized monoclonal anti-C1s antibody

Jakob Mühlbacher (1), Bernd Jilma (2), Markus Wahrmann (3), Lena Marinova (3), Farsad Eskandary (3), James C. Gilbert (4), Sandip Panischer (4), Georg A. Böhmig (3)

(1) Department of Surgery, Medical University Vienna, Vienna, Austria, (2) Department of Clinical Pharmacology, Medical University Vienna, Vienna, Austria (3) Department of Medicine III, Medical University Vienna, Vienna, Austria (4) True North Therapeutics, Inc., South San Francisco, CA, USA

jakob.muehlbacher@meduniwien.ac.at

138A Role of GABAergic interneurons to prefrontal cortex network activity during a rule-switching task

Lagoun, S.1 , Lagler, M.1, Ozdemir, A. T1., and Klausberger, T.1,2

1: Department of Cognitive Neurobiology, Center for Brain Research, Medical University of Vienna, Austria

2: MRC Anatomical Neuropharmacology Unit, Oxford University, Oxford

sabria.lagoun@meduniwien.ac.at

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-shifting 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.

139 Elucidating Otoprotective Mechanisms of Steroids by Systemic Application of the Selective Glucocorticoid Receptor Agonist Compound-A: a Potential Alternative to Dexamethasone?

Zhu,Chengjing (1), Landegger,Lukas (1)*, Honeder,Clemens (1), Schöpper,Hanna (2), Engleder,Elisabeth (3), Gabor,Franz (3), Gstoettner,Wolfgang (1), Arnoldner,Christoph (1)

(1) Medical University of Vienna, (2) University of Veterinary Medicine Vienna, (3) University of Vienna

zhuzhuxaz@gmail.com

Glucocorticoids have been widely used for many inner ear disorders for decades. However, their otoprotective mechanism is poorly understood and some of the concomitant side effects can lead to severe health complications (e.g. hyperglycemia or osteoporosis). Selective glucocorticoid receptor agonists (SEGRAs) are a novel class of drugs, which are thought to reduce side effects and demonstrate similar pharmacological potency relative to glucocorticoids. To our knowledge, no research has been performed related to its otoprotective capacities. Therefore, we aim to directly compare the effects of a potent steroid, dexamethasone, and one of the SEGRAs, Compound-A, in an animal model of noise trauma.40 pigmented guinea pigs were enrolled. Experimental agents included Compound A (3 mg/kg or 1 mg/kg), dexamethasone (1 mg/kg) as

gold standard and water (1 mg/kg) as negative control. Animals received an intraperitoneal injection once daily for 10 consecutive days. After 5 applications, each animal was exposed to broadband noise at 115 dB for three hours. Hearing thresholds were determined by recording auditory brainstem responses (ABR) immediately after exposure, as well as on day 1, 3, 7, 14, 21 and 28. After euthanasia, ears were extracted for histological study.Compared to the negative control and dexamethasone, the two dosage regimens of the novel compound failed to preserve auditory thresholds after noise exposure with statistical significance. Histological analyses of hair cell and spiral ganglion neuron counts confirmed the physiological results. Although the current study revealed that Compound A does not seem to have the same otoprotective capacities as dexamethasone, our results give interesting insights into the function of glucocorticoids: apparently, the dimerization of the receptor is essential for their mechanism of action in the inner ear.Funding: This study was supported by the Austrian Science Fund (FWF grant P 24260-B19) and MedEl Austria.

140 It runs in the family: genetic and non-genetic predictors of hemispheric dominance

Schuler, A.-L. (1), Kasprian, G.(2), Seidl, R.(1), Kienast, P.(2), Lackner, S.(1), Gruber, G.M.(3), Prayer, D.(2), Bartha-Doering, L.(1)

(1) Department of Pediatrics and Adolescent Medicine, Medical University of Vienna, (2) Department of Biomedical Imaging and Image-guided Therapy, Medical University of Vienna, (3) Center for Anatomy and Cell Biology, Department of Anatomy, Medical University of Vienna

anna-lisa.schuler@meduniwien.ac.at

It is widely believed that functional lateralization to one cerebral hemisphere is an important marker for healthy cognitive development. Although evidence suggests shared genetics for handedness and hemispheric language dominance, the exact neurobiological preconditions of language lateralization are not explained exhaustively. The aim of this study is to investigate the predictive value of genetic precursors, early biomarkers and environmental influences in terms of language lateralization. 16 healthy children aged 6-13, (10 monolingual, 6 multilingual) who had prenatal magnetic resonance imaging (MRI), participated in this study. To assess functional language lateralization, they performed a word definition paradigm inside the MR scanner. Factors used related to functional lateralization were prenatal superior temporal sulcus (STS) depth, handedness (EHI), familial left-handedness and number of languages spoken. Language lateralization in the frontal lobe and temporal lobe significantly correlated with familial left-handedness, indicating children with left-handedness in their family as being more right lateralized. Association between fetal STS and frontal lobe functional asymmetry revealed five distinct clusters. Cluster one, consisting of monolingual children with familial right-handedness, exposed a strong correlation between fetal STS depth and functional lateralization in frontal lobe.

Languages spoken significantly correlated with functional lateralization in the frontal and temporal lobes, indicating stronger right-lateralization in multilingual children. This study provides new evidence to the theory of partial pleiotropy of handedness and language lateralization. It features the factor familial left-handedness as strong predictor of language lateralization. In addition, prenatal cortical folding patterns serve as a precursor of language lateralization for genetic right-handers if no environmental factors influence this process of lateralization.

141 Resting-state functional connectivity changes in Male-to-Female transsexuals after hormonal treatment

Seiger, R (1),Hahn, A (1), Sladky, R (2), Kranz, GS (1), Ganger, S (1), Hummer, A (2), Spies, M (1), Windischberger, C (2), Kasper, S (1), Lanzenberger, R (1)

(1) Department of Psychiatry and Psychotherapy, Medical University of Vienna, Austria, (2) MR Centre of Excellence, Center for Medical Physics and Biomedical Engineering, Medical University of Vienna, Austria

rene.seiger@meduniwien.ac.at

It has been shown that cross-sex hormone therapy in Male-to-Female (MtF) transsexuals led to a significant decrease in serotonin transporter (SERT) binding in the anterior cingulate cortex (ACC) after a 4 months treatment period with estradiol and anti-androgens. On the other hand, studies already showed sex differences in resting-state functional connectivity (rsFC), which may be related to fluctuating hormonal levels. Here we investigated whether these hormonal induced changes in the ACC are accompanied by changes in rsFC between the baseline measurement and after 4 months. The PET analysis has already been carried out in a prior study. For the rsFC analysis, a subsample of the PET study was included and comprised 15 MtFs (29±6.8 years), measured before and after a 4-months period of hormonal treatment. MRI measurements were conducted at 7T using a 32-channel head coil. An EPI sequence was used (TE/TR=23/1400ms, 32 axial slices, voxel size= 1.5x1.5x2mm). Data were corrected for slice timing effects and movement, spatially normalized and smoothed). RsFC was computed using the ACC as seed region and connectivity maps were z-transformed. Comparing rsFC changes between the baseline and the treatment period using the ACC as seed region results showed significant increases after 4 months ($p<0.05$ corrected, $t=3.4$, cluster size=370). These increases were found in the postcentral gyrus, precentral gyrus, the supplementary motor area, cuneus, precuneus, the superior parietal cortex and in the right insula. This study showed increased rsFC metrics in MtF subjects after hormonal treatment between the ACC and widespread regions in the brain including somatosensory and motor areas as well as the insula. Hence, decreased SERT binding in the ACC was accompanied by connectivity increases in the motor network and networks

related to body representation and awareness indicating hormonal induced alterations related to these domains.

142 Brain- activity- cycling in amplitude- integrated EEG – what do we see in polysomnography?

Lisa Schmidt (1),Zsofia Rona (2)*, Vito Giordano (2)*, Tobias Werther (3)*, Sampsa Vanhatalo (4)**, Angelika Berger (1)*, Katrin Klebermass-Schrehof (1)*

* MUW, Department of Pediatrics and Adolescent Medicine, ** University of Helsinki, Department of Neurophysiology and Neurobiology

lisa.schmidt@meduniwien.ac.at

BACKGROUNDBrain activity cycling (BAC) is described in very premature infants in aEEG. Recent data shows a correlation to the CNS integrity and therefor it could be a sensitive marker for later outcome. The aim of this study was to analyze the emergence of cyclicity in premature infants within predefined periods of “active state” (AS) and “quiet state” (QS) by aEEG with polysomnography. Additionally an automated EEG-analysis (NLEO- algorithm) and a conventional visual EEG analysis were done.METHODSPolysomnographies were preformed every second week for 3 hours until term equivalent age in preterm infants less than 28 weeks of gestation. With simultaneously acquired aEEG (C3-C4) 10-minute epochs of QS and AS were selected for analysis. The interburst- intervals (IBI) and burst durations (BD) in AS and QS were visually analyzed and by using an NLEO- algorithm calculating SAT (=spontaneous activity transients) percentage.RESULTS109 polysomnographies of 44 preterm infants were analyzed. The mean IBIs decreased in QS and AS with increasing gestational age (GA) (QS 7.38 to 2 (r2 0,226), AS 5 to 2 (r2 0,101)) and the mean BD increased in QS and AS with increasing GA (QS 5.69 to 22 (r2 0,149), AS 10.83 to 26 (r2 0,035)). The SAT% also increased with increasing GA and from QS to AS periods.CONCLUSIONIn our data we see BAC within aEEG and polysomnography in very preterm infants and a correlation between QS and AS with increasing GA. New methods and reference values for early GA will make assessment easier and clinically applicable.

143 Evaluating Midazolam as an active placebo in a new acute pain model

Prosenz, J. (1), Gustorff, B. (2)

(1) Medical University of Vienna;(2) Wilhelminen hospital

julian.prosenz@meduniwien.ac.at

144 Do transfemoral amputees face worse consequences? Physical and psychological differences in transtibial and transfemural amputees during rehabilitation

Armbruster. C. (1), Domayer, SE. (2)

Medical University of Vienna, Orthopedic Rehabilitation Center Zicksee

chaska.armbruster@gmail.com

Background: During orthopedic surgery, efforts are made to preserve as much of the lower limb as possible, as the amputation level has an influence on future mobility and rehabilitation extent. It is presumed that patients with transfemoral (TF) amputation might face more difficulties in regaining physical health and mobility than patients with transtibial (TT) amputation. This might also concur with a decrease in health-related quality of life (HRQoL) and mental health.Material and Methods: 33 TT amputees and 17 TF amputees were assessed at admission (T1) and at discharge (T2) during their stay at an orthopedic rehabilitation center regarding mobility, activities of daily living (ADLs), prosthesis use, pain, HRQoL, depression and body-image.Results: Both groups enhanced their performances in the different measurements significantly from T1 to T2. However, TT amputees performed better in all conditions right from the start. Furthermore, TF amputees reported significantly higher pain sensation, especially phantom limb pain. Both groups improved most regarding their physical health. Increased physical health was a predictor for increased mental health.Conclusion: Results concur with former findings that TF amputees face more difficulties in regaining mobility and physical health. The results underline the importance of physical health for an improved mental health.

145 RELATIONSHIP NURTURING SOCIAL INTERACTION – CONSIDERING THE EFFECTS OF AFFECT- REGULATION AND AFFECT- EXPERIENCE ON WORKING WITH DEPRESSED PATIENTS

Datz, F.(1), Madanolgu, S. (1), Löffler-Stastka, H. (1)

Medical University Vienna

felicitas.datz@meduniwien.ac.at

Social interaction describes individuals reacting and influencing each other, building a relationship. Psychotherapeutic schools agree that the relationship between therapist and patient is crucial to treatment success. In crisis intervention a prompt alliance building is required. Interaction can be an unconscious, automatic part of the relationship forming process. To date few studies and theoretical tracts focus on social interaction in conjunction with the therapeutic relationship. We analyzed 234 tape-recorded therapy sessions of 100 depressed patients from the Munich Psychotherapy Study (Huber, 2012), a comparative quasi-experimental study of psychoanalysis (PA), psychodynamic therapy (PD) and Cognitive behavioral therapy (CBT). The study aimed to analyze therapeutic interactions in order to yield a correlation between the patient's positive affect and the therapist's positive countertransference, forming a relationship nurturing interaction pattern. External validity was optimized by examining non-manualized and representative psychotherapies under the conditions of daily clinical practice. The detected social interaction structures indicate that a positive patient affect is followed by a therapist's benevolent countertransference reaction (and vice versa). The confirmed social interaction pattern implies that not only a positive affect is related to a relationship nurturing countertransference reaction, but that the same countertransference entails a positive affect.

146 Consequences of patient representation and role exposure of Simulation Patients

Butollo, M., A.,

Medical University of Vienna

maria.butollo@meduniwien.ac.at

Background: Students get a specific education in medical communication by Simulation Patients (SP) to learn sensibility for and knowledge of communication procedures in difficult problems before they work with real patients. One argument for the use of SPs is the higher resilience of the SPs compared to real patients. Roleplay increases mental health in psychotherapy. Healing experience or awareness takes place in an "as if - reality" and the play is used as a cure. If the role play of healthy behavior increases health, there is a good case to believe decrease of mental state through the play of patient roles like SPs do. The aim of the study was to explore the negative impact of stressful roles on SPs and the SPs strategies to get out of the roles after play.Method: 22 SPs of Medical University of Vienna participated in half structured interviews. They were asked about feelings after role play, technique to get in and out of the role and consequences of patient representation. The interviews were transcribed and contend analyzed. Categories were found in a deductive way.Results: Consequences of patient representation on SPs mental state can be biographical incidents, frequency of representations in a row, behavior of students and teachers during roleplay and SPs actor technique. To decrease that effect SPs used relaxation methods like PMR, motoric methods, verbal techniques, imaginative method or reflection about the role play. Discussion: To prevent the negative impact of stressful patient

representation we assessed SPs techniques to get out of the patient role. Those techniques have to be trained well. People have difficulties to distance from their work in different fields. To increase work life balance there can be use of SPs techniques for workers in health systems as well.

147 Effect of BDNF Val66Met on suicidal behaviour in affective disorders.

Carlberg, Laura, Klemens Kienesberger (1), Patrick Swoboda1, Birgit Ludwig(1), Romina Koller(1), Alexandra Bernegger(1), Nestor Kapusta(2), Martin Aigner(3), Helmuth Haslacher(4), Michaela Schmöger(5), Siegfried Kasper(1) and Alexandra Schosser(1,6),*

(1) Department of Psychiatry and Psychotherapy, Medical University of Vienna, Vienna, Austria, (2) Department of Psychoanalysis and Psychotherapy, Medical University of Vienna, Vienna, Austria, (3) Federal Hospital Tulln, Department of Psychiatry, Tulln, Austria. (4) Department of Laboratory Medicine, Medical University of Vienna, Vienna, Austria. (5) Department of Neurology, Medical University of Vienna, Vienna, Austria, (6) Zentrum für Seelische Gesundheit LEOpoldau, Vienna, Austria.

laura.carlberg@meduniwien.ac.at

148 Improvement of diagnostic process in lesions of the coronal part of front teeth

Bulbuk, Oleksandr

Dentistry Department of Ivano-Frankivsk National Medical University, Ukraine

bulbuk77@gmail.com

The most common and early form of lesion of tooth-jaw system are the defects of teeth crowns of different origin. The crown of the teeth and the methods of treatments are changed depending on the size and location of the defect. The work is aimed at improving the methodology for determining the amount of hard tooth tissue defects in the frontal area, that will help to develop a code coronal part destruction of front teeth.The aim of the study: optimize the diagnostic process in the treatment of lesions of coronal part of frontal teeth through development the methods for determining the volume of hard tooth tissue defects in the frontal area.Materials and methods: we were conducted an experimental research of different size and localization of hard tissue defects of frontal teeth on the diagnostic models and 20 extracted teeth with preserved coronal part.Results: the research of diagnostic models which were proposed by us, we determined the volumetric percentage relationship of defects of the teeth to the volume of crown for different defects of frontal teeth.Conclusions. Methodology of determining the amount of hard tissue

defects in the frontal group of teeth by making creamy substance with insulin syringe on the investigated teeth model or prints of the data frontal teeth will correctly execute a detailed diagnosis of lesions coronal front teeth and to monitor the reasonableness conducted orthopedic treatment .

149 Non-cytotoxic alternatives to SDS for cartilage decellularization

Schneider, C. (1), Lehmann, J. (3), Oberbauer, E. (1), Hildner, F. (2), van Osch, G. (3), Redl, H. (1), Wolbank, S. (1), Nürnberger, S. (1,4)

(1) Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Vienna, Austria, (2) Red Cross Blood Transfusion Service of Upper Austria, Linz, Austria, (3) Erasmus Medical Center, Rotterdam, The Netherlands, (4) Department of Trauma Surgery, Medical University, Vienna, Austria

cornelia.schneider@trauma.lbg.ac.at

INTRODUCTION: In articular cartilage, full removal of the reagents used in the process of decellularization is often challenging due to its dense structure. Therefore harmful substances potentially remaining in the tissue could affect cells when seeded. The negative effect of sodium dodecyl sulphate (SDS), one of the most commonly used decellularizing agents, has been described in literature. The aim of this study is to find an alternative which decellularizes articular cartilage without negatively affecting cellular attachment and viability.METHODS: Human articular cartilage was harvested from femoral heads. Ionic and non-ionic detergents, oxidizing agents as well as acids and bases were tested as alternative to SDS. Cell removal and the effect on the extracellular matrix were evaluated via biochemical assays and histological examination. Seeding tests with adipose derived stem/stromal cells (ASCs) were conducted.RESULTS: Sodium hydroxide was established as most effective for glycosaminoglycan (GAG) reduction and hydrochloric acid proved to efficiently decellularize the scaffold. A combination of both was therefore subjected to the cytotoxicity test, which showed good adhesion of cells to the scaffold surface, as opposed to the poor results obtained by the SDS-treated scaffolds. However, sodium hydroxide was shown in to influence collagen type II in the outer layers of the scaffold. Therefore a screening of various enzymes is planned, aiming to find a reagent for targeted GAG depletion which leaves the other matrix components intact.DISCUSSION & CONCLUSIONS: A protocol consisting of freeze/thaw cycles, osmotic shock and treatment with sodium hydroxide followed by hydrochloric acid was established, achieving successful decellularization and depletion of GAGs within one week. While SDS had a strongly negative effect on cell viability and adherence, cells form a homogenous monolayer on scaffolds treated with the new protocol.

150 Investigation of bisphosphonate -induced toxicity on dental pulp stem cells

Solmaz Pourgonabadi

Department of Oral Biology, Medical University of Vienna, Austria

solmaz.pourgonabadi@meduniwien.ac.at

151 Effects of Hypoxia and Hypoxia Mimetic Agents on Sclerostin and Dickkopf-1 production in Dental pulp-derived cells

Janjić, K. (1, 2), Cvikl, B. (1, 2, 3), Moritz, A. (1, 2), Agis, H. (1, 2)

(1) University Clinic of Dentistry, Department of Conservative Dentistry and Periodontology, Medical University of Vienna, Austria (2) Austrian Cluster for Tissue Regeneration, Vienna, Austria (3) Department of Preventive, Restorative and Pediatric Dentistry, University of Bern, Switzerland

n0949196@students.meduniwien.ac.at

Tooth trauma and avulsion can cause hypoxic conditions in the dental pulp. Preservation of cell vitality therefore depends on the pulp's regeneration capacity under hypoxia and its involving molecular mechanisms. Sclerostin (SOST) and Dickkopf (DKK)-1 are key inhibitors of Wnt signaling, a pathway that stimulates regeneration in various oral tissues and that could also play a role in dental pulp regeneration. The aim of this project was to assess the effects of hypoxia and the hypoxia mimetic agent L-mimosine (L-MIM) on the production of SOST and DKK-1 in the dental pulp. Dental pulp-derived cells were harvested from extracted retromolars with informed consent. Cells were stimulated with hypoxia or L-MIM in each cell culture model. Untreated cells under normoxic conditions served as controls. All experiments were conducted in two different in vitro models: a 2D monolayer and a 3D spheroid cell culture. Cell vitality was determined by a Resazurin toxicity test. Gene expression of SOST and DKK-1 was analyzed by qPCR. On protein level, SOST and DKK-1 were analyzed by ELISA. Cell vitality under stimulation with hypoxia or L-MIM was confirmed in 2D and 3D models by the Resazurin toxicity test. After stimulation with hypoxia or L-MIM gene expression of SOST and DKK-1 was reduced relative to the control in both models. Also on protein level reduction of both inhibitors was shown in both models. Hypoxia and L-MIM inhibit mRNA and protein synthesis of the Wnt-signaling inhibitors SOST and DKK-1 in a 2D and 3D model of dental pulp-derived cells. These data help to a better understanding of the cellular responses to hypoxia in the dental pulp, as well as the effects of hypoxia mimetic agents. Future in vivo studies will reveal the role of SOST and DKK-1 in dental pulp regeneration. This work was funded by the European Society of Endodontology (ESE).

152 Collagen barrier membrane induces pro-inflammatory phenomenon in RAW-264.7 cells as murine bone marrow derived macrophages

Solmaz Pourgonabadi,Reinhard Gruber

Department of Oral Biology, Medical University of Vienna, Austria -Department of Preventive, Restorative and Pediatric Dentistry, School of Dental Medicine, University of Bern, Switzerland

solmaz.pourgonabadi@meduniwien.ac.at

Collagen barrier membrane (CBM) has been utilized in oral and periodontal surgery because of its material was proven in supporting wound healing. Pro-inflammatory reaction is necessary for wound healing process. In our previous study, we have reported that CBM decrease osteoclastogenesis in murine bone marrow derived macrophages. Bone marrow cells also give rise to macrophages, a heterogeneous cell population involved in pro-inflammatory and wound healing. Activation of pro-(M1) and anti-(M2) inflammatory macrophages arise from Lipopolysaccharide (LPS) and IL-4 (interlukine-4), respectively. Here in, the impact of CBM on programming bone marrow cells into either M1 or M2 macrophages is unclear. In this study, we investigated weather CBM effects on the process of macrophage polarization in vitro based on RAW-264.7 cells as murine bone marrow derived macrophages. CBMs provoke an activation of the M1 phenotype, characterized by a surge of IL12, also slight increase in IL6, NOS2 as pro-inflammatory based on real-time gene expression analysis. Ym1, MR as anti-inflammatory genes have mild increase on M2 phenotype by CBMs. Our preliminary results suggest that CBMs induced pro-inflammatory phenomenon however, it raises both of M1 and M2 genes in RAW-264.7 cells.

153 T2-relaxometry of in-vivo compressed knee cartilage at 7T

Roehrich, S. (1), Szomolanyi, P. (1), Schreiner, M. (2), Juras, V. (1), Trattnig, S. (1)

(1) Vienna,AT,Medical University of Vienna,Department of Biomedical Imaging and Image-guided Therapy High Field MR Centre, Vienna,AT, (2) Medical University of Vienna,Department of Orthopedic Surgery.

sebastian.roehrich@meduniwien.ac.at

Water content and collagen structure of hyaline cartilage can be evaluated through MRI T2-mapping. Additionally, an alteration in T2-values can be measured when load is applied,

reflecting the biomechanical state of cartilage. Therefore, we assessed T2-values during a compression cycle at 7 Tesla [T] with a 3D triple echo stead state sequence [3D-TESS] to utilize the high resolution and fast image-acquisition thereof. The knees of 10 healthy subjects (age 24 to 33 years) were measured under unloading-loading-unloading conditions (25kg) via an MRI-compatible compression device and T2 maps were analyzed by drawing regions of interest [ROIs] in anterior and posterior parts of femoral and tibial cartilage. Mean T2 values of superficial and deep zones of cartilage were compared with Student's t-test and among different loading conditions with repeated measurements ANOVA (Bonferroni post hoc test).In average, T2 values of all ROIs were 50% higher in the superficial zone than in the deep zone (femoral: $p<.0001$; tibial: $p<.001$). Adjacent to cartilage under compression, ROIs showed increased T2 values: anterior femoral superficial and deep ROIs (15%, $p=.003$; 11%, $p=.017$), anterior tibial superficial and deep ROIs (16%, $p= .003$; 13%, $p=.02$), and tibial posterior superficial ROI (17%, $p=.025$). The other adjacent ROIs showed also a trend towards increased T2 values, however, this was not significant.Contrary to cartilage adjacent to compressed regions, all ROIs in the centre of applied load showed a trend towards decreased T2 values, however, without significance.Biomechanical alterations in in-vivo knee cartilage can be reliably measured with 3D-TESS at 7T at much shorter acquisition times (1:58min) than in previous studies (7:35min). Physiological distribution of water under compression in knee cartilage may lead to increased T2 values adjacent to compressed cartilage and to decreased T2 values in the region of directly compressed cartilage.

154 Chemoresistance of SW1353 Chondrosarcoma Cells Towards Cisplatin Is Partly Dependant On Autophagy

Reumann S (1),(2), Shogren KL(2), Yaszemski MJ(2), Windhager R(1), Maran A(2)

(1) Department of Orthopedics, Medical University of Vienna, Vienna, Austria; (2) Department of Orthopedics, Mayo Clinic, Rochester, MN

s.reumann@yahoo.com

Chondrosarcoma is a rare tumor entity, nevertheless accounting for 16-20% of all bone sarcomas. The most striking characteristic of chondrosarcoma is its's relatively high radio- and chemoresistance leaving surgery as the only curative treatment option. Considering that the survival rates for chondrosarcoma have stayed the same for the last decades there is a great demand for a curative systemic treatment. Therefor the underlying mechanisms of chemoresistance in chondrosarcoma need to be investigated. As found previously the anti-tumor effect of 2-Methoxyestradiol in chondrosarcoma cells can be enhanced via inhibition of autophagy. This study aims to investigate the synergistic effect of Cisplatin + Bafilomycin. In order to evaluate cell viability in SW1353 cells treated with Veh, 2-ME +/- Bafilomycin andCisplatin +/- Bafilomycin we used MTS-assays. Ultrastructural analysis of SW1353 cells

treated as mentioned above was performed as well as Western Blot analysis of LC3 I & II to determine whether Cisplatin induces autophagy in SW1353 chondrosarcoma cellls.Interestingly we found that Cisplatin only slightly induces autophagy in SW1353 chondrosarcoma cells, however we were able to detect autophagosomes via Transmission Electron Microscopy. Additionally we found that the synergistic effect of Cisplatin and Bafilomycin is relatively small compared to our previous findings with 2-Methoxyestradiol. These observations lead to the conclusion that Cisplatin does not induce autophagy as efficiently as 2-ME in SW1353 chondrosarcoma cells. It strengthens our previous hypothesis that the autophagy might be a mechanism of chemoresistancy in SW1353 chondrosarcoma cells treated with Cisplatin.

155 High-resolution axonal bundle (fascicle) assessment and triple-echo steady-state T2 mapping of the median nerve at 7Tesla: preliminary experience

Georg Riegler,Drlicek G. (1), Kronnerwetter C. (1), Heule R. (2), Bieri O. (2), Bodner G. (1), Lieba-Samal D. (3), Trattnig S (1)

(1): 1.Medical University of Vienna, Department of Biomedical Imaging and Image-guided Therapy; (2): 2.University of Basel Hospital, Department of Radiology, Division of Radiological Physics; (3) 3.Medical University of Vienna, Department of Neurology

georg.riegler@meduniwien.ac.at

156 Early Biomechanical Properties of Anatomical Glenoid Reconstruction with the J-Bone-Graft for Anterior Glenoid Bone-Loss

Leo Pauzenberger, Dyrna F, Obopilwe E, Cote MP, Heuberer PR, Anderl W, Mazzocca AD.

Dept. of Orthopaedic Surgery, St. Vincent Hospital Vienna, Austria

leo.pauzenberger@bhs.at

157 Normal mandibular growth and diagnosis of micrognathia at prenatal MRI

Nemec SF, Brugger PC, Weber M, Bartsch B, Bettelheim D, Gruber M, Prayer D

MUW, Department of Biomedical Imaging and Image-guided Therapy

ursula.nemec@meduniwien.ac.at

158 Effects of phylloquinone and magnesium on ATDC5 prechondrocytes

Raimann, A (1) Javanmardi A. (1), Sagmeister, S (1), Haeusler G. (1)

Medical University of Vienna

adalbert.raimann@meduniwien.ac.at

159 Jawbone remodeling with different implant surfaces: Histomorphometric study in mini-pigs

Wenjuan Zhou (1, 2) Stefan Tangl (1), Sonja Kuderer (1), Michael Müller-Kern (3), Zhonghao Liu (2)*, Xiaohui Rausch-Fan (3)*

(1) Karl Donath Laboratory for Hard Tissue and Biomaterial Research, Department of Oral Surgery, University Clinic of Dentistry, Medical university of Vienna, Vienna, Austria, (2) Department of Implant Dentistry, Yantai Stomatological Hospital, Binzhou Medical University, Yantai, China, (3) Competent Center of Periodontal Research, Division of Conservative Dentistry and Periodontology, University Clinic of Dentistry, Medical university of Vienna, Vienna, Austria

zhouwenjuan1004@163.com

160 Saliva supports the formation of pro-inflammatory in murine bone marrow cultures

Solmaz Pourgonabadi, Heinz-Dieter Müller - Reinhard Gruber

Department of Oral Biology, Medical University of Vienna, Austria

solmaz.pourgonabadi@meduniwien.ac.at

160A The peroneus brevis footprint and its relevance in the surgical treatment of the Jones Fracture

M. Willegger[1], N. Seyidova[2], R. Windhager[1], R. Schuh[1], L. Hirtler[3]

madeleine.willegger@meduniwien.ac.at

160B Hospital linen as a potential vector for multi-drug resistant microorganisms

Zatorska, B., Dorrer, S., Diab-El Schahawi, M., Presterl, E

beata.zatorska@meduniwien.ac.at

Background: Outbreaks with infectious bacterial microorganisms in intensive care units pose an important infection control problem. Transient colonization of human skin, the gastrointestinal tract and also the environment by mdros (multi-drug resistant microorganisms) in the hospital setting is not unusual, but if infection control guidelines are not respected cross-contamination with subsequent nosocomial infection can occur. Hospital clothing as a possible vector of mdro should be excluded. Aim: This study evaluates the survival of different bacteria species on fabrics used as hospital gowns.

Methods: 5x5 cm large pieces of cloth from freshly cleaned hospital linen were prepared for further analysis. Each of those materials was artificially inoculated with 1 mL of a 10 5 bacterial suspensions using a sterile cotton swab (4mm / 14cm Hartmann □). The following bacterial species were analyzed: S. epidermidis DSM 269, E.hirae DSM 3320, E.coli ATCC25922, P. aeruginosa ATCC 27853, K.pneumonie 07-288-001 / 02 and A.baumanii (4MRGN). Contact cultures were taken directly from the hospital linen. Plates were incubated for 48 h at 35 °C. The survival of the various bacterial species on moistened textiles (300 mL 0,9% NaCl) and on textiles dried for 1 hour in the laminar air-flow was determined by recording the number of colony forming units (CFU). All experiments were repeated in triplicate

Results: Growth of Gram-positive bacteria S. epidermidis, E. hirae and A. baumannii as the sole representative of the Gram-negative bacteria was found both on wet fabrics as well as after 1 hour drying. No growth of Gram-negative bacteria after 1 hour drying was found and only scattered colonies of K.pneumoniae on dried fabrics.

Discussion: Bacteria can survive on humid hospital linen at least for 1 hour. Gram-negative bacteria can be effectively killed by drying with the exception of A. baumannii whose ability to survive on dry surfaces corresponds to that of Gram-positive bacteria.

161 ICAM-1 Binding Rhinoviruses A89 and B14 Uncoat in Different Endosomal Compartments

Ganjian, H.

Medical University of Vienna

halehganjian85@gmail.com

Human rhinovirus (HRV) -A89 and HRV-B14 bind to and are internalized by intercellular adhesion molecule 1 (ICAM-1); as demonstrated earlier, the RNA genome of HRV-B14 penetrates into the cytoplasm from endosomal compartments of the lysosomal pathway. Here we show, by immunofluorescence microscopy, that HRV-A89 but not HRV-B14 colocalizes with transferrin in the perinuclear recycling compartment (PNRC). Applying drugs differentially interfering with endosomal recycling and with the pathway to lysosomes, we demonstrate that these two major group HRVs productively uncoat in distinct endosomal compartments. Overexpression of constitutively active (Rab11-GTP) and dominant-negative (Rab11-GDP) Rab11 mutants revealed that uncoating of HRV-A89 depends on functional Rab-11. Thus, two ICAM-1 binding HRVs are routed into distinct endosomal compartments for productive uncoating.

162 Association between asthma control and quality of life in asthmatic pregnant women

Nasrin Fazel[a], , Michael Kundi [b], Erika Jensem-Jarolim[c], Isabella Maria Pali- Schöllc, Asghar Kazemzadeha, Mojtaba Fattahi [a], Habibollah Esmailyd, Roya Akbarzadeh a, Raheleh Ahmadia ,

[a] University of Medical Sciences, Sabzevar, Iran

b Center for Public Health, Medical University Vienna, Vienna, Austria,

c Institute for Pathophysiology and Allergy Research, Medical University Vienna, Vienna, Austria, and

d Department of Biostatistics & Epidemiology, Neonatal Research Center, Mashhad University of Medical Sciences, Mashad, Iran

n1242078@students.meduniwien.ac.at

Background: One of the most prevalent chronic diseases in the world is asthma. we periodically assessed, as part of the routine care programs, the symptoms, limitations and asthma control of such cohorts.

Methods: We conducted a prospective study from August 2014 to June 2015, on pregnant asthmatic patients. To assess the degree of asthma control we used the Asthma Control (ACQ) and Asthma Quality of Life (AQLQ) questionnaires and the standards recognized by the Global Initiative for Asthma (GINA). Mann-whitney test Descriptive and Fisher Exact Test analyses were performed, $p<.05$ was measured for statistical significance.

Results: We evaluated a total of 43 non-pregnant controls, 35 years or older, with intermittent, mild, moderate or severe persistent asthma with 12 (38.2%), 10 (, 29.4%), 8 (23.5%), and 3 (8.8%) pregnant asthmatic patients, respectively. There was a significant relationship between AQLQ and ACQ ($p< 0. 001$). The two groups had a significant difference with regard to

limitations (p=0.002). The two groups also had a significant difference in the rate of asthma symptoms when waking up in the morning (p <0.001). Overall, we found that most pregnant asthmatics (67.6%) had a favorable AQLQ versus a somewhat favorable AQLQ (32.4%).

Conclusions: The majority of pregnant asthmatics have a favorable AQLQ. Moreover, we observed a high degree of concordance between ACQ and AQLQ. The outcomes of this study reinforce the importance of controlling asthma control for maintaining quality of life in gravidity.

Key Words: asthma ,quality of life , pregnancy

163 DEVELOPMENT OF A SYSTEM FOR SUPERVISED TRAINING AT HOME WITH KINECT V2

Scherer, M. (1) Unterbrunner, A. (2), Riess, B. (2), Kafka P.(2)

(1) Medical University of Vienna, (2) UAS Technikum Wien

scherer@technikum-wien.at

Therapy of patients can take long periods of time. It is important to promote sustained motivation during long rehabilitation phases. Live feedback and playful applications enhance the compliance of users. Through gamification of the therapeutic process the injured patients' encouragement and the therapeutic success can be increased. Low-cost sensors like the Microsoft Kinect are a useful way to implement inexpensive feedback applications to strengthen flexibility, perseverance and speed in the clinic and home environment. For this project the newest version of the Microsoft Kinect sensor was used and implemented into a rehabilitation system. In contrast to recent available systems, this one compares the patient's movements to individually recorded references of the patient himself, instead of standard data from literature. This system provides a new and individualized approach which opens new doors for rehabilitation at home. During clinical stay the reference files are recorded under supervision of a therapist. This files consists of data from predefined exercises. During work out at home, the algorithm compares the actual movements with the reference data and gives individual live feedback. In order to increase the patient‘s compliance, the exercises were implemented as games or similar to games with different levels of difficulty. This enables the patient to recognize his rehabilitation progress more easily during the training at home. This playful approach combined with a proper gamification concept ensures a constant high level of motivation for all types of patients. The correct execution of an exercise is one of the most important aspects during therapy. Otherwise joints, ligament and muscles may be damaged or injured. The system aims for patients of all ages recovering from injuries. This innovative system is able to increase strength, fitness and speed of reaction.scherer@technikum-wien.at

164 Current and Speed Response of a Left Ventricular Assist Device in Single and Dual Stator Configuration

Maw, M. (1,2,3), Aigner, P. (1,3), Schima, H. (1,2,3), Moscato, F. (1,3), Aigner, P. (1,3), Schima, H. (1,2,3), Moscato, F. (1,3)

(1) Center for Medical Physics and Biomedical Engineering, Medical University of Vienna; (2) Department of Cardiac Surgery, Medical University of Vienna; (3) Ludwig-Boltzmann-Cluster for Cardiovascular Research, Vienna; Austria.

martin.maw@meduniwien.ac.at

Objectives: The housing that encases the impeller of the HVAD left ventricular assist device contains two stators, situated at the top and bottom. For detailed investigation of the pump dynamics and the influence on flow estimation, a detailed knowledge of the interaction between the two stators is desirable. Therefore a setup was established, which allowed the characterization of the HVAD operation in single and dual stator configuration.Methods: The temperature-controlled mock-loop setup consisted of a positive displacement pump, an air trapped reservoir and a controllable pinch valve. This facilitated modulation of inlet and outlet pressure. Water/glycerol mixtures constituted the working fluids. Static and dynamic measurements were conducted. During dynamic measurements the displacement pump produced a sinusoidal 0.5-20Hz pressure head sweep. Results: The static and dynamic responses of the single and dual stator configurations were identified at working points between 0-12L/min and 0-200mmHg. In both, the static and the dynamic tests, motor current uptake was up to 5% and 20% higher for bottom and top single stator mode respectively, compared to dual stator mode. Current peak dynamic response was shifted from 4.9 ±0.2Hz in dual stator configuration to 3.0±0.2 Hz in single stator configuration. Current and speed showed resonant behaviour at lowest and highest speeds, which are, however, outside of the clinically used range. Conclusion: The characterization of the current and speed response of the HVAD could be performed over a wide frequency range. In combination with effective filtering, it can be used to further optimize a highly-responsive flow estimator for different regular and extreme working conditions.

165 Development of a closed-loop control system for upper limb prosthesis integrating EMG and position feedback

Kast, C. (1) Aramphianlert, W.(1), Krenn, M.(1), Hofer, C.(3,2), Aszmann, O.C.(2), Mayr, W.(1)

(1) Center for Medical Physics and Biomedical Engineering, Medical University of Vienna, Vienna, Austria (2) Christian Doppler Laboratory for Restoration of Extremity Function,

Division of Plastic and Reconstructive Surgery, Department of Surgery, Medical University of Vienna, Vienna, Austria (3) Otto Bock Healthcare Products GmbH, Vienna, Austria

christoph.kast@meduniwien.ac.at

An active prosthetic arm needs reliable control inputs for accurate and safe positioning in the three dimensional space. Here, we present a modular concept for a device, which integrates a control system based on electromyography (EMG) and inertia sensors for position feedback. The hardware design is based on three different modules: an eight-channel EMG-module, a motion capture module and a synchronization module. Recorded data are analyzed in a connected personal computer that allows to perform complex decisions and control algorithms fast enough for real-time control of the prosthesis actuators.The recording system is capable of combining up to eight acquisition modules, each with eight bipolar inputs, and data transfer via USB-interface. Important for control applications as well as experimental multi-channel recording, is reliable synchronization of all acquired signals. Different modules are coordinated by a separated clock module, which provides the time-base for the microcontrollers and delivers trigger signals. The developed system ensures a synchronization error smaller than 10µs within 10s for simultaneous signal recording. The EMG signals of up to 64 bipolar channels are processed and filtered in groups of 8 inputs by the analog front-end circuit, an ADS1299 analog-to-digital converter (Texas Instruments Inc., Dallas, TX, USA). Recording is not limited to EMG; other electrical bio-signals, e.g. electrooculogram (EOG) or electroencephalogram (EEG), could serve as alternative control sources, like EEG in brain-computer interface approaches.The system provides a flexible design, which is of great importance for the intended primary application for advanced real-time control of a prosthetic arm and hand. The modules are compact enough to be carried for mobile use. The USB-interface allows uncomplicated interaction with standard computers and gives at the same time easy access to a simple and reliable power supply.

166 Brain Atrophy Measurement Employing Non-Rigid Registration Using Free Form Deformation Based on De-Boor Control Points

Ghodrati, M. (1)*, Schima, H.(2), Fatemizadeh, E. (3)

(1) Center for Medical Physics and Biomedical Engineering, Medical University of Vienna (2) Islamic Azad University Science and Research Branch, Biomedical Engineering, Tehran, Iran

mojgan.ghodrati@meduniwien.ac.at

Brain Atrophy is one of the common markers in many neuro-degenerative processes such as Alzheimer’s disease and Multiple Sclerosis. Several methods have been proposed for brain atrophy measurement, among which Jacobian Integration (JI) has proven to be one of the most

precise. This method uses the non-rigid Free Form Deformation (FFD) registration based on B-Spline basis functions in which the control points are the parameters. Measuring the exact amount of small atrophies occurring within the brain, requires very precise methods. This paper aims to optimize the JI algorithm. Some changes in the image registration algorithm are made, in particular replacing De-Boor points with B-spline control points, leading to a new curve and new basis functions (D-Spline basis functions) which have properties identical to B-Spline basis functions. Some interpolation examples using the two spline methods were employed to compare their accuracy. Using the D-Spline method, the error in interpolating curves decreased by more than two-fold and the error in interpolating surfaces by almost four-fold. The evaluation of the two spline methods in atrophy measurement was done in two steps. First, 7 MRI images from BrainWeb were compared to similar images uniformly reduced by 3% in each dimension to approximate atrophy typical of an AD patient over one year. The two methods were applied to these images. The results suggested that the D-Spline method was able to recognize 2.25% and the B-Spline method about 2% of the imposed volume reduction. As a second step, atrophy was measured in 20 images of patients diagnosed with Alzheimer's disease obtained from the ADNI website. After volume reduction measurement the B-Spline method showed 2.067% and the D-Spline method 2.21% volume reduction. Results show that the D-Spline method performs better in both curve and surface interpolation. In comparing the two methods in brain atrophy measurement, the D-Spline method was significantly more accurate.

167 Investigation of energy efficiency of different waveforms and electrode arrangements to activate motor neurones

Schmoll M. (1)(2) Jarvis J.C. (2)

(1) Medical University of Vienna, (2) Liverpool John Moores University martin.schmoll@meduniwien.ac.at

168 Optimizing Trial Designs for Targeted Therapies - A Decision Theoretic Approach Comparing Sponsor and Public Health Perspectives

Ondra, T. (1) Jobjörnsson, S.(2), Beckman, R.A.(3), Burman, C.F.(4), König, F.(1), Stallard, N.(5) , Posch, M.(1)

(1) Medical University of Vienna, (2) Chalmers University of Technology, (3) Georgetown University, (4) AstraZeneca R&D, (5) Warwick Medical School

thomas.ondra@meduniwien.ac.at

An important objective in the development of targeted therapies is the identification of the population where the treatment under consideration has a positive benefit risk balance.We consider the setting where the efficacy of a treatment is tested in an overall population and/or a pre-specified subgroup. Based on a decision theoretic framework we derive optimized clinical trial designs by maximizing expected utility functions. The utility functions quantify benefits and costs from both sponsor and public health perspectives.Features to be optimized include the sample size, the population the trial is performed in (the full population or the targeted subgroup only) as well as the characteristics of the underlying multiple testing procedure. The considered sponsor's utility function accounts for the hypothesis testing procedure and models the expected revenue as well as the cost of the pivotal clinical trials. In addition, we consider a public health utility function that accounts for the actual health benefit of the treatment for the population. Examples of optimized trial designs are given for several scenarios and optimal trials for the sponsor's and public health utilities are compared.

169 Group Sequential and Adaptive Designs with Multiple Endpoints

Urach, S. Posch, M.

Medical University of Vienna

susanne.urach@meduniwien.ac.at

Analyses of multiple endpoints in clinical trials often use alpha exhaustive tests relying on the joint multivariate distribution which either presume known correlations or estimates based on all data accumulated so far. As misspecification of the correlation can have a large impact on the type I error rate, it is often proposed to use the most conservative adjustment across all possible correlations. As it is a waste of information to take the maximum over all possible values of the correlation, Berger and Boos suggest a method to restrict the maximization of the nuisance parameter to a confidence set, but still control the type I error rate of the trial. A method based on the confidence interval approach by Berger and Boos can be used to exactly control the type I error rate while still using estimates of the correlations between endpoints in the interim analyses of a group sequential trial. As the arctan hyperbolic transformation of the sample correlation coefficient only approximates the normal distribution we investigate the robustness of the method for small sample sizes. Consequences on error rates and power when the asymptotic assumption of a multivariate normal distribution for the test statistics (based on z-tests with known variance) doesn’t hold but t-tests are performed are investigated. Additionally we extended the methodology to more than one nuisance parameter and applied it on tests for three endpoints where three correlation coefficients need to be estimated. Boundaries are improved for hierarchical designs with one primary and two equally important secondary hypotheses (semi-hierarchical approach) or a secondary and a tertiary hypothesis (totally hierarchical approach). Consequences of choosing one of these approaches on different power definitions are compared

under different parameter settings. Results are illustrated using a clinical trial example in a rare disease.

170 Implementation of Exploratory Subgroup Analysis in Clinical Trials: A Interactive and Practical Approach

Ballarini, N. Graf, A., Koenig, F., Posch, M.

Medical University of Vienna

nicolas.ballarini@meduniwien.ac.at

Subgroup analyses in clinical trials comparing two treatments are pursued to identify biomarkers that are prognostic, predictive or both. While prognostic biomarkers are associated with the outcome (independently of which treatment the patients receive), predictive biomarkers identify patients which are more likely to benefit from an experimental treatment. The identification of biomarkers is especially challenging when the number of biomarkers to investigate is large, since standard estimators are typically biased. We developed a simulation app to quantify the bias resulting from simple subgroup analyses as well as subgroup identification via the application of model selection procedures. This comprehensive tool allows exploring models with dichotomous and continuous endpoints as well as biomarkers, varied biomarker correlation structures, model selection procedures and estimation techniques. We give results for a wide range of scenarios including the case of small samples, where the subgroup sizes may be highly variable. The app has been implemented as an open source R-package and can optionally be accessed through a Shiny GUI.

171 A comparison of the shrinkage effects of penalized and post-estimation shrinkage methods in logistic regression

Kammer, M. (1,2) Dunkler, D. (1), Heinze, G. (1)

(1) Section for Clinical Biometrics, Medical University of Vienna, (2) Department of Medicine I, Medical University of Vienna

michael.kammer@meduniwien.ac.at

Shrinkage refers to the phenomenon that predictions from a statistical model tend to be too extreme when applied to new data, which was not used to develop the model. This effect is

especially problematic in the case of small sample size or many predictors, as often seen in clinical practice. Penalized regression and post-estimation shrinkage methods can be utilized to correct for shrinkage. By pulling regression coefficient estimates towards zero, they introduce a (small) bias in order to reduce the overall predictive error. These fundamentally different shrinkage methods are implemented, e.g., in the R packages glmnet and shrink, respectively.In a simulation study, which allows full control over the population characteristics, we compare how well these methods anticipate and correct for shrinkage in logistic regression problems typically encountered in biostatistical practice. Sample sizes and events per variable [EPV] are chosen such that, in principle, shrinkage of estimates improves predictive accuracy. As penalized approaches we apply ridge regression and LASSO. We compare them to global and parameterwise shrinkage methods applied after unpenalized estimation. Here, we also evaluate the impact of backward variable selection. Results indicate that in situations with few EPV penalized methods tend to pessimism, i.e. anticipate too much shrinkage, while post-estimation methods are more optimistic about the optimal amount of coefficient shrinkage. This makes penalized methods the safer choice for pure prediction purposes, but nevertheless, post-estimation methods might be preferable if better control over the model building process is needed or the interpretation of the coefficients is relevant.Furthermore, a prediction model for preterm birth risk is used to discuss practical aspects of applying shrinkage methods in real life.

172 Non-linear B-spline image registration for three-dimensional reconstruction of brain tissue

Anner, P. (1) Passecker, J. (2), Klausberger, T. (3), Dorffner, G. (4)

Medical University of Vienna

philip.anner@meduniwien.ac.at

Generating three dimensional (3D) models of paraformaldehyde-fixed sections of brain tissue is a major task in current research. The models can be used for spatial identification of entities such as cell somata or blood vessels. Histological entities are cut in thin slices and cells can be further identified by immunohistological labeling. The slices are usually 3D scanned with a confocal microscope resulting in a z-stack. Image registration is the process of aligning, transforming, and merging the z-stacks to a 3D model. We evaluated linear and non-linear image registration techniques within the rats' prefrontal cortex. The cutting process introduced inhomogenities on the upper and lower borders of the brain slices (70 um thickness), which resulted in local loss of information. This was accounted for by applying maximum intensity projection (MIP). As a measure of similarity between the images of the consecutive slices we used the squared intensity differences of the pixels. The measure was enhanced by manually selected reference points. Linear transformations and affine transformations were evaluated, but resulted in non-satisfactory results. Non-linear distortions caused by fixation, cutting and the mounting on the glass probe of the tissue have to be accounted in the image registration technique. A simulation

study was performed to assess the image registration techniques based on images of one physical slice. Images with a gap of 21nm were selected and the image to be registered was spatially deformed. The result of the image registration process was then compared to the original (non-deformed) image. Three dimensional histological reconstructions using linear and B-spline transformations were generated. The image registration was assessed visually by focusing on entities (cell somata, blood vessels and dendrites) that appeared on consecutive sections. Using B-spline transformations we could remove the distortions in order to achieve a consistent 3D model.

173 How to incorporate safety for efficacy testing in multi-arm clinical trials

Hlavin, G. Koenig, F.

Medical University of Vienna / Center for Medical Statistics, Informatics, and Intelligent Systems / Section for Medical Statistics

gerald.hlavin@meduniwien.ac.at

174 Interim analyses incorporating short- and long-term endpoints for binary correlated outcomes

Niewczas, J. (1) Kunz, C. (2), König, F. (3)

Medical University of Vienna, Lancaster University

julia.niewczas@meduniwien.ac.at

175 Comprehensive CRISPR screens for mapping heme-induced programmed cell death

Quattrone, F.(1,2), Martins, R.(1,2), Rebsamen M.(1), Girardi, E.(1), Superti-Furga, G.(1), Knapp, S.(1,2).

(1) CeMM, Research Center for Molecular Medicine of the Austrian Academy of Sciences, Austria (2) Department of Medicine I, Research Laboratory of Infection Biology, Medical University of Vienna, Austria

Fquattrone@cemm.oeaw.ac.at

Heme is an essential molecule in life and serves as a prosthetic group for a variety of proteins. These are involved in diverse functions such as oxygen transport, respiration and signal transduction. The presence of ferrous iron confers heme the ability to handle electrons and bind divalent gas molecules, but conversely, renders it an extremely reactive molecule. Therefore, under homeostasis, the levels of free heme, are tightly controlled by scavenging proteins and catalyzing enzymes. These defense mechanisms are geared towards curbing the pro-oxidant effects of free heme in order to prevent damage to cell components and cell death. However, upon severe hemolysis, the rupture of red blood cells results in the release of vast amounts of heme from hemoglobin greatly exceeding the scavenging and catabolic capacity of the organism and severe damage to exposed tissues occurs. The majority of the heme-triggered cytotoxic effects have been attributed to the pro-oxidant nature of the molecule. Nevertheless, increasing evidence indicates that, instead, heme has specific targets mediating its cytotoxicity, since different tissues undergo different programmed cell death events. We aim at studying the molecular mechanisms underlying heme cytotoxicity, as well as mechanisms of resistance to free heme. For this, we will employ a systematic approach of genome-wide genetic perturbation screens to generate loss and gain of function mutations in human cell lines. We will use both the GeCKO v2 CRISPR library and the Cas9-SAM CRISPR system, to create pools of knock-out or gene-overexpressing clones. These clones will be subjected to selective pressure with heme and the surviving clones will be sequenced in order to identify which targets and/or pathways mediate heme cytotoxicity or resistance against free heme. These results will be analyzed using bioinformatic tools to pinpoint the cell-damage/-death pathways triggered by heme and validated using in vitro and in vivo models.

176 Advantage of large fieldofview highspeed OCTAngiography in clinical application

Laurin Ginner (1)Cedric Blatter (2), Lara M. Wurster (1,4), Abhishek Kumar (1,4), Reinhard Told (3), Andreas Pollreisz (3), Ursula Schmidt-Erfurth (3) and Rainer A. Leitgeb (1,4)

(1)Center for Medical Physics and Biomedical Engineering, Medical University of Vienna, (2) Dermatology, Massachusetts General Hospital; (3)Ophthalmology and Optometry, Medical University of Vienna; (4) Christian Doppler Laboratory (OPTRAMED), Medical University of Vienna

laurin.ginner@meduniwien.ac.at

Demonstrating the improvement of OCT Angiography (OCTA) image quality for wide-field (16°) imaging of clinically relevant cases, by increasing the A-scan rate to 400kHz exploiting the spectral splitting (SP) method in swept source OCT (SPOCTA). 3D OCTA data were acquired with three different A-scan rate settings: 70 kHz (spectral domain OCT, AngioVue, 800nm central wavelength), 100 kHz (swept source OCT (prototype), 1060nm) and 400 kHz (swept source OCT (prototype), with spectral splitting (SP)). The data have been acquired in cases of

age-related macular degeneration (AMD) patients, including cases of choroidal neovascularization (CNV) and geographic atrophy (GA). The swept source OCT system utilizes spectral splitting (SP) to double the lateral sampling and thereby increasing the A-scan rate to 400 kHz. We compare the results of representative patient measurements in case of CNV and GA with all three systems. The increase in A-scan rate to 400kHz after applying SP in swept source OCT allows extending the FOV from 8 to 16deg by maintaining the same lateral sampling rate and thus essentially the same quality in terms of microvascular contrast and SNR. We determined an average SNR increase of 4 dB by changing from 100 kHz to 400 kHz keeping the same FOV. Large filed of view is in particular important for extended lesions as in the case of GA. The method of SP allows employing a commercially available source operating at 200kHz and operating the OCTA at virtually 400kHz. Our results based on SP OCTA demonstrate that a higher A-scan rate enhances the diagnostic capabilities by providing better signal contrast and structural detail. Especially in case of GA, where the affected area in patients is commonly larger than the FOV of a standard OCT A modality (8°). The presented SPOCTA system is currently the fastest OCT angiography system based on a commercially available swept source.

177 Advantage of large fieldofview highspeed OCTAngiography in clinical application

Fialová, S. (1) Augustin, M.(2), Knopf, C.(3), Pircher, M.(4), Schmetterer, L.(5), Hitzenberger, CK.(6), Baumann, B.(7)

Medical University of Vienna

stanislava.fialova@meduniwien.ac.at

178 Multi-channel depth encoded swept source joint aperture Doppler optical coherence tomography

Wartak, A. (1) Haindl, R. (1), Trasischker, W. (1), Baumann, B. (1), Pircher, M. (1), Hitzenberger, C. K. (1)

Center for Medical Physics and Biomedical Engineering, Medical University of Vienna, Austria

andreas.wartak@meduniwien.ac.at

Retinal blood flow is a critical factor concerning the development of various severe eye diseases such as glaucoma, diabetic retinopathy and central/branch retinal vein occlusion. Especially in glaucoma, alterations in retinal blood flow seem to be linked very closely to an increased intraocular pressure, which gives rise to the discussion of the disease’s actual origin.We present a

multi-channel depth encoded swept source joint aperture Doppler optical coherence tomography (JA-D-OCT) system for absolute flow velocity measurements in vitro – in a flow phantom (glass capillary) – as well as in vivo – to quantify human retinal blood flow. Our experimental swept source Mach-Zehnder interferometer setup features one active illumination channel and two passive detection channels. These linearly independent oriented measurement directions allow for reconstruction of the absolute 3D-velocity-vector without any prior knowledge about flow orientation. Via the implementation of only one active illumination channel we are no longer forced to divide illumination power among three beams to meet laser safety requirements. The in vitro measurement results correspond very nicely to the expected flow velocity values. For the in vivo measurements we evaluated retinal vessel bifurcations regarding their respective flow rates. Due to the principle of mass conservation, total in- and outflow at a vessel bifurcation need to match, which could be demonstrated for three evaluated bifurcations in three eyes of three healthy human volunteers.Quantification of the total retinal blood flow per eye – which will be the next step – will help study above mentioned eye diseases more thoroughly. This work is funded by the FWF grant #P26553-N20.

179 Sensitivity and dose rate effects of MAGAT and MAGIC polymer gels for 3D-dosimetry in radiation therapy with different oxygen scavenger concentrations

Khan,M. (1) Heileman, G.(2). Georg, D. (2,3), and Berg, A. (1)

(1) MR Centre of Excellence, Center for Biomedical Engineering and Physics, Medical University of Vienna, Waehringerstrasse 13, A-1090, Vienna, Austria (2) Department of Radiation Oncology, Medical University of Vienna/AKH Vienna, Austria (3) Christian Doppler Laboratory for Medical Radiation Research for Radiation Oncology, Medical University of Vienna, Austria

n1442190@students.meduniwien.ac.at

Introduction: Magnetic resonance imaging [MRI] based polymer gel dosimetry [PGD] offers three dimensional [3-D] information on dose distributions to verify planned and actual dose in radiation therapy. Many of the proposed polymer gels [PG] have shown a dose response dependency on dose rate. Most often Methacrylic Acid Gel Inititated by Copper [MAGIC] type gels are used. For very sensitive dosimetry, Tetrakis Hydroxymethyl Phosphonium Chloride [THPC] is used as an Oxygen Scavenger [OS] in methacrylic acid gelatin [MAGAT] type gels. The aim of this study was to report the dose response for a range of dose rates with respect to different OS concentrations. Methods: Polymer gels were manufactured in-house using gelatin, water, methacrylic acid and different OS based on THPC or ascorbic acid. A linear accelerator and X-ray machine [YXLON] were used for irradiation to cover a large range of different dose rates. T2-weighted MR scans of the gels were performed on a Siemens 7T scanner. From the resulting T2-values, R2 maps were generated, which are related to the dose levels, obtained from

the cross-calibration with an ionization chamber. Results: For MAGAT, in the high dose regime, the dose rate had a higher effect on dose response than in the low dose regime. The sensitivity [-1Gy-1 at 0.3 Gy/min to decrease to 1.67 Gy-1 at 25 Gy/min. MAGIC with an increased concentration of OS showed reduced dose sensitivity, which dropped from 0.3s-1Gy-1 to 0.1s-1Gy-1 at dose rates of 4 Gy/min, typical in clinical routine. With increasing ascorbic acid concentration the linear dose range extended from 50 up to 65 Gy and advantageously the dose response was not as sensitive on dose rate.Summary/Conclusion: MAGAT was found to be most sensitive, but showed the highest dose rate dependency. An increased OS concentration in MAGIC reduced the dose rate effect. For dosimetric applications no or minimal dose rate dependency is important.

180 Retinotopic organisation of the primary visual cortex in patients with central vs peripheral retinal dysfunction

Hummer, A. (1); Ledolter, A. (2); Ritter, M. (2); Tik, M. (1); Schmidt-Erfurth, U.(2); Windischberger, C.(1)

(1) Centre of Medical Physics and Biomedical Engineering, Medical University of Vienna, (2) Department of Ophthalmology and Optometry, Medical University of Vienna

allan.hummer@meduniwien.ac.at

Purpose: The Blood-oxygenation-level dependent (BOLD) contrast measured by functional Magnetic Resonance Imaging (fMRI) in the primary visual cortex (V1) represents neuronal activity as a reaction to the stimulation of the central retinal regions (“population receptive fields” (pRF)). Patients with inherited retinal dystrophies develop an absolute functional deficit at the level of retina as a result of photoreceptors’ death. As consequences of such retinal dysfunctions are not fully determined at the cortical level, we link microperimetry and fMRI results to investigate the influence on cortical activity.Methods: We used BOLD fMRI (3T Siemens Trio; TE/TR=30/1500ms, voxel size 1x1x1mm, CMRR multiband sequence with 28 slices) in 5 patients with Stargardt disease (SD) and 5 patients with Retinitis Pigmentosa (RP). Visual stimulus for fMRI was a moving, flickering checkerboard bar crossing the screen in eight different directions and covering the central 20° visual angle. BOLD response was modelled for each voxel using a 2D Gaussian pRF model as implemented in mrVista (Stanford University, Stanford, CA). Functional loss on the retinal level was determined using microperimetry MP1 (Nidek, Italy) in all patients.Results: Eccentricity parameters of the pRF model showed the expected mapping, but no significant BOLD response was obtained near the posterior pole of the visual cortex for SD and anterior parts of the visual cortex for RP patients, implying a lack of stimulus-related cortical activity. The regions where the pRF model explained more than 10% BOLD signal variance corresponded to a large extend to preserved retinal function as measured by microperimetry.Conclusions: We demonstrated that retinotopic maps for SD patients showed

central scotomas, while activation corresponding to preserved central retina function was found in RP patients. Retinotopic mapping therefore also complements perimetry in patients suffering from retinal dysfunction.

181 Comparison of fALFF at 3 Tesla and 7 Tesla

Woletz, M.(1) Tik, M.(1), Lamm, C.(2), Windischberger, C.(1)

Medical University Vienna, University of Vienna

michael.woletz@meduniwien.ac.at

Low-frequency oscillations in resting state fMRI data sets are the basis for most resting-state functional connectivity studies. A parameter for the magnitude of these low-frequency oscillations is an index named amplitude of low-frequency fluctuations (ALFF) and the normalised versions thereof (fALFF). These values were used for comparison between healthy controls and clinical populations in several studies. Here we examine the influence of field strength on fALFF values in healthy subjects by comparing data sets acquired at 3T and 7T. Resting state scans were acquired at 7T and 3T using the CMRR multiband EPI sequence (7T: 1.4s/23ms TR/TE, 1.5x1.5x1.25 mm^3, multiband factor 3, 3T: 1.8s/33ms TR/TE, 1.5x1.5x2mm^3, multiband factor 3). All scans lasted for 6 minutes with the subjects keeping their eyes open and fixed at a fixation cross.Scans were despiked, corrected for slice-timing, distortion and motion, normalised to the MNI-152 space nuisance regressed. fALFF maps were computed using Matlab's Discrete Fourier Transform to extract the amplitudes of the frequencies from 0.01 to 0.1 Hz and normalised to the sum of all amplitudes larger than 0 Hz. Additionally, the 7T were adapted to the 3T TR by matching the maximum frequency in the denominator. All maps were smoothed using a 3mm FWHM Gaussian Kernel. For a region of interest analysis grey and white matter masks were created using SPM12.The calculated maps show that the 7T fALFF maps appear very similar to the 3T fALFF maps, but with reduced overall values. Adapting the denominator to the 3T frequency range will eliminate these differences. ROI analysis showed an increase by a factor of 1.88 in the contrast between grey and white matter for 7T.We conclude that fALFF values are consistent across field strengths but the enhanced contrast at 7T. In combination with a recent study on the temporal stability of fALFF maps our results support the idea of fALFF values as potential biomarkers in health and disease.

182 Total retinal blood flow in healthy and glaucomatous human eyes measured with three beam Doppler optical coherence tomography

Haindl, R. (1), Wartak, A. (1), Trasischker, W. (1), Holzer, S. (2), Baumann, B. (1), Pircher, M. (1), Vass, C. (2), Christoph K. Hitzenberger (1)

(1) Center for Medical Physics and Biomedical Engineering, Medical University of Vienna, Austria, (2) Department of Ophthalmology, General Hospital and Medical University of Vienna, Austria

richard.haindl@meduniwien.ac.at

183 Investigation of different workload contributions of gastrocnemius medialis and soleus during plantar flexion with variable knee angles using localized multivoxel 31P MRS at 7 Tesla

Niess, F. (1) Fiedler, G.B. (1), Schmid, A.I. (1), Goluch, S. (2), Kriegl, R. (1), Wolzt, M. (3), Moser, E. (1), Meyerspeer, M. (1)

(1) Vienna,AT,Medical University Vienna,Center of Medical Physics and Biomedical Engineering, (2) Vienna,AT,Medical University of Vienna,Department of Internal Medicine III, (3) Vienna,AT,Medical University Vienna,Department of Clinical Pharmacology

fabian.niess@meduniwien.ac.at

184 Presence of subretinal fluid at baseline preserves from photoreceptor alterations in diabetic macular edema and cystoid macular edema due to central retinal vein occlusion

Ana-Maria Philip, Podkowinski Dominika; Pablik Eleonore; Montuoro Alessio; Waldstein Sebastian M.; Gerendas Bianca; Schmidt-Erfurth Ursula

Medical Universtity of Vienna

ana-maria.philip@meduniwien.ac.at

185 The foveal shape is not predictive of visual acuity and treatment response in macular edema due to retinal vein occlusion

Podkowinski Dominika, Jing Wu, Ana-Maria Glodan, Bianca S. Gerendas, Alessio Montuoro, Roland Leitner, Christian Simader, Sebastian M. Waldstein, Ursula Schmidt-Erfurth

Medical University of Vienna

dominika.podkowinski@meduniwien.ac.at

186 Molecular imaging of the antigen recognition dynamics in CD8+ cytotoxic T-cells

Kraller M., René Platzer, Paul Spechtl, Johannes B. Huppa and Hannes Stockinger

Molecular Immunology Unit, Institute for Hygiene and Applied Immunology, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna

markus.kraller@meduniwien.ac.at

Cytotoxic T-cells (CTLs) are of paramount importance for the immune defense against viruses and tumors. Remarkably, CTLs can detect with their low affinity T-cell antigen receptors (TCRs) the presence of even a single MHC class I molecule loaded with a specific antigenic peptide (pMHCI) among thousands of structurally related yet non-stimulatory pMHCs (Purbhoo et al. 2004). How they achieve this is not clear but appears to depend at least in part on the special binding conditions within the special constraints of the immunological synapse, the area of contact between a T-cell and an antigen presenting cell. Here receptors and their ligands are not only pre-oriented, but they are often enriched in specific membrane domains and also subjected to cellular forces. To relate these cell biological parameters to T-cell antigen sensitivity in a more comprehensive manner we are monitoring TCR-pMHC binding in nascent synapses with the use of molecular imaging modalities. We confront TCR transgenic CTLs with a glass-supported lipid bilayer functionalized with pMHCI, adhesion and co-stimulatory molecules. This allows us to conduct (single molecule) measurements in noise-attenuated Total Internal Reflection (TIRF) mode, to control for ligand composition and density to quantitate their specific influence on TCR-pMHCI binding and TCR-proximal downstream signaling. We also plan to assess the role of CD8 co-receptor engagement with the use of pMHCI mutants, which are deficient in CD8 binding. We expect to gain novel insights into cell biological and molecular processes underlying the phenomenal sensitivity of CTLs towards antigen.

187 Testing the impact of neutralization of cleaved osteopontin on human liver-cancer-cell tumor

Igenicity

Nardo, A.D. Grün, N.G.(1), Stoiber-Sakaguchi, D.(2), Mikulits, W.(3), Zeyda, M.(1), Stulnig, T.M.(1)

(1)Christian Doppler Laboratory for Cardio-Metabolic Immunotherapy and Clinical Division of Endocrinology and Metabolism, Department of Medicine III, Medical University of Vienna,

Währinger Gürtel 18-20, 1090, Vienna, Austria. (2)Ludwig Boltzmann Institute for Cancer Research , Vienna , Austria. (3)Department of Medicine I, Institute of Cancer Research, Medical University of Vienna, Vienna, Austria.

alexander.nardo@meduniwien.ac.at

Background: Hepatocellular carcinoma (HCC) is the most common form of liver cancer and is the cause of approximately 1 million deaths yearly. Osteopontin (OPN) is involved in promotion of cancer cells by regulating various facets of tumor progression such as cell proliferation, invasion, angiogenesis and metastasis. Overexpression of OPN has been found in a variety of cancers, including liver cancer. While OPN´s isoforms and their blockades are well investigated in cancer research, little is known about OPN´s cleavage products. Since MMPs and thrombin have been observed at the tumor site in several animal models, and since it has been shown that these cleavage products can be even more active than the full length OPN, we hypothesize that the treatment with our in-house produced antibodies against mmp- and thrombin-cleaved OPN will have a negative effect on the cancer-cell-line tumor progression and/or metastatization in human xenograft models. Methods: three strong-OPN-expressing and one OPN-non-expressing human liver cancer cell lines will be selected via qRT-PCR analyses and used in the further xenograft models of tumorigenicity and metastasis. In both studies, four treatment groups will be defined: CMIP005 21-5-4 (against thr-cOPN), CMIP003 9-3 (against mmp-cOPN), IgG CTRL, PBS. Tumor growth and establishment of micro-metastases in distal organs are the primary and secondary end points, respectively.Conclusions: the data obtained will elucidate whether the cleaved forms of OPN play a role in the pathophysiology of liver cancer and if the immunotargeting of them can be an effective therapy for HCC. This work is supported by the CCHD doctoral program of the FWF (W1205-B09), and the Federal Ministry of Economy, Family and Youth and the National Foundation for Research, Technology and Development (to T.M.S.).

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

Bhat, S. (1)*

Hasenhuetl, PS.(1), Sandtner, W.(1), Freissmuth, M.(1)

(1) Department of Pharmacology, Medical University of Vienna, Austria

shreyas.bhat@meduniwien.ac.at

The serotonin transporter (SERT) and the dopamine transporter (DAT) terminate synaptic transmission by reuptake of their cognate neurotransmitter from the synaptic cleft back into presynaptic neurons. SERT and DAT also have a rich pharmacology binding to a plethora of

illicit substances derived from amphetamine and cathinone. Amphetamines and substituted amphetamines induce serotonin and dopamine efflux from presynaptic neurons through SERT and DAT respectively. However some compounds of the phenethylamine library (PAL) exhibit low efficacy in inducing efflux when compared to amphetamines through unknown mechanisms. We hypothesize that this is due to PAL compounds trapping the transporters in specific conformational states during the transport cycle. We approached this hypothesis using an electrophysiological approach involving recording of different electric currents carried through transporters during substrate transport in HEK293 cells stably expressing SERT: peak current reflects substrate induced charge movement; steady-state current indicates inward facing conformation visited by the transporter during the conformational cycle. Currents induced by partial releaser PAL-1045 were compared to those induced by complete releasers (PAL-287, PAL-1046 and para-Chloroamphetamine [pCA]) and serotonin (5-HT). Reduced steady state amplitudes of currents with increasing concentrations of PAL compounds suggest that PALs bind with high affinity to both the inward and outward facing conformations of SERT. Slower recovery of 5-HT induced peak currents on PAL-1045 application, when compared to the full releasers, also argues for longer dwell-time of PAL-1045 in its binding site which precludes intracellular serotonin binding and efflux. Taken together, our observations provide evidence for a mechanism resulting in low-efficacy substrate efflux through SERT. The results have implications for developing low-efficacy releasers as therapeutic agents for addiction therapy.

189 How antigen is presented to sensitize T-cells

Platzer R.(1) Brameshuber M.(2), Schütz G.J.(2), Stockinger H.(1), Huppa J.B.(1)

(1) Institute for Hygiene and Applied Immunology, Center for Pathophysiology, Infectiology, Immunology, Medical University of Vienna. (2) Institute for Applied Physics, Vienna University of Technology.

rene.platzer@meduniwien.ac.at

T-cell antigen recognition is based on a transient interaction of T-cells with professional antigen presenting cells (APC). This unique cell contact – termed the immunological synapse – is shaped by the quality and quantity of molecular interactions between specific T-cell antigen receptors (TCRs) on the T-cell site and peptide-loaded major histocompatibility complexes (pMHC) on the APC. T-cells react to antigen in a strikingly sensitive manner : they respond to the presence of a single stimulatory pMHC even though TCR-pMHC binding, when measured in solution, is generally weak, and antigenic pMHCs are often vastly outnumbered by structurally similar but non-stimulatory pMHCs. Here we present evidence that the nanoscale organization of pMHCs within APC plasma membranes affects TCR recognition dynamics considerably. Initially, tracking of single pMHC molecules and fitting to two distinct populations exhibited slower and faster moving fractions. Simultaneously applied high-speed photo activated localization

microscopy exposed areas with a high density of pMHCs within the membrane of living APCs that could very well accelerate antigen recognition by the scanning T-cell. To study this in a defined manner we are currently examining the functional relevance of pMHC compartmentalization with the use of functionalized lipid bilayers (SLBs), which serve as surrogate APC. To correlate lateral diffusion and membrane organization of pMHCs to TCR-ligand engagement and TCR-proximal signaling, we are devising methodologies to monitor simultaneously synaptic TCR-pMHC binding and downstream signaling in T-cells contacting SLBs and living APCs.

190 Probing the immuno-regulatory function of Lipocalin 2 in pulmonary diseases

Watzenböck, M. (1) Drobits, B. (1,2), Starkl, P. (1,2), Gorki, T. (1,2), Lakovits, K (1,2), Hladik, A (1,2) and Knapp, S (1,2)

(1) CeMM, Research Center for Molecular Medicine of the Austrian Academy of Sciences, Austria; (2) Department of Medicine I/Research Laboratory of Infection Biology, Medical University of Vienna, Austria

martin.watzenboeck@meduniwien.ac.at

Lipocalin 2 (LCN2) is a small secreted protein which has been implicated in a variety of biological processes. By binding a subset of bacterial siderophores, it limits iron acquisition of bacterial strains that depend on siderophores. In LCN2-deficient mice, this leads to increased susceptibility to infections with siderophore-dependent bacteria such as Escherichia coli and Klebsiella pneumoniae. Furthermore LCN2 is involved in immuno-modulatory mechanisms as it deactivates macrophages and impairs bacterial clearance in a mouse model of pneumonia caused by Streptococcus pneumoniae (a siderophore independent pathogen). In this project, we aim to investigate the influence of LCN2 on antiviral immune responses and immunopathology during influenza infection. We found that pulmonary LCN2 is induced in WT C57BL/6 mice following influenza virus infection. LCN2-deficient mice display increased weight loss, enlarged mediastinal lymph nodes (MLNs) and prolonged histologically assayed tissue destruction compared to WT controls. Viral load (measured by qPCR) was not increased in LCN2-deficient mice. FACS analysis of lungs of infected LCN2-deficient mice and WT controls revealed increased T-cell counts, both relative and absolute, in LCN2-deficient mice. However, both in-vitro proliferation studies and T-cells counts in the lungs of infected WT/LCN2-KO mixed bone-marrow chimeric mice did not show an increased intrinsic proliferative potential of LCN2-deficient T-cells. Upon co-culture with OT1-T-cells, we saw that CD103+ dendritic cells from MLNs of LCN2-deficient mice infected with an ova-bearing influenza virus induce stronger T-cell proliferation when compared to dendritic cells from WT controls. In summary, our results indicate that LCN2 controls viral immune responses to prevent exaggerated tissue damage.

Ongoing studies are focused on the question how immune cells sense LCN2 and if recombinant LCN2 can be used therapeutically to fine tune inflammatory responses.

191 Kv7 channels: Potential targets for antinociceptive action of Paracetamol

Sutirtha Ray Isabella Salzer, Stefan Boehm

Department of Neurophysiology and Neuropharmacology, Center for Physiology and Pharmacology, Medical University of Vienna, Waehringerstrasse 13a, A-1090 Vienna, Austria

sutirtha.ray@meduniwien.ac.at

Background: Paracetamol/ Acetaminophen (APAP) is a widely used analgesic whose mechanism of action remains controversial. Enhancement of currents through Kv7 potassium channels in dorsal root ganglion (DRG) neurons reduces excitability thereby providing analgesia. Therefore, effects of APAP and its metabolites on Kv7 channels were investigated.Methods: Currents through Kv7.2, 7.3, and 7.5 expressed in tsA201 cells were recorded using the perforated patch-clamp technique. Results: Currents through recombinant homomeric Kv7.2 and Kv7.5 channels were increased by NAPQI by upto 250% and 400% respectively while those through Kv7.3 homomers were decreased down to 40%; both effects were irreversible and concentration-dependent. With Kv7.2/7.3 and Kv7.3/7.5 heteromers, currents were enhanced to 120% and 250% respectively in a concentration dependent manner up to 3 µM NAPQI and depressed at higher concentrations, the effect being irreversible. On application of 3 µM NAPQI for 10 minutes, the Q2 CCC150-152AAA mutant had an inhibition down to 20% while the Q2 C492A mutant showed an enhancement up to 250%, the effects were irreversible. Paracetamol (1 mM) and AM404 (10 µM) had no effect on homomeric Kv7.2 and Kv7.3 currents.Conclusion: These results indicate that the mechanism of action of paracetamol could be explained by the enhancement of Kv7 currents by its metabolite NAPQI.

192 Interaction of platelets with dendritic cells

Bucsaiova, M., Mannhalter, C.

Department of Laboratory Medicine, Medical University of Vienna

martina.bucsaiova@meduniwien.ac.at

Background: The fundamental function of platelets in haemostasis is well-established and undisputed. However, it is increasingly evident that platelets also have other physiological roles. They have been shown to participate in immune functions, inflammation, tissue regeneration, lymphatic development, as well as various pathological conditions such as cancer and atherosclerosis. High relevance of communication between platelets and other cell types, such as endothelial cells, neutrophils and monocytes but also others, has been demonstrated experimentally. However, the interaction with dendritic cells remains under-explored. Indeed, a handful of studies indicate that there is both, cell-to-cell and interaction with soluble components. Yet the findings are incomplete and contradictory. Therefore, we decided to study this in more detail. Methods: Washed platelets were activated by TRAP or convulxin, or were un-activated. Immature dendritic cells were differentiated from blood monocytes by culturing them with GM-CSF and IL-4. The two cells types were co-incubated in an allogeneic culture for 2 days, with or without very low concentrations of LPS. Results: Both, resting and activated platelets were found to associate with dendritic cells. Activation of platelets by both agonists lead to a higher proportion of binding between dendritic cells and platelets. However, the effect of platelets on dendritic cell maturation, as measured by the expression of maturation markers on their surface, was unclear under the conditions used. We are currently in the process to investigate the type of the interaction. Conclusion: Our findings suggest an influence of platelets on dendritic cells. We plan to elucidate whether this interaction then influences the polarization of T cells following an interaction with dendritic cells. Future experiments will aim to determine if platelets are capable of shuttling antigens to dendritic cells, as has been previously shown in mice.

193 Ischemic postconditioning modulates focal adhesion signaling pathway in porcine model

Lukovic, D. (1), Pavo, N.(2), Zlabinger, K.(3), Gugerell, A.(4), Winkler,J.(5), Giricz, Z.(6), Baranyai, T.(7), Ferdinandy, P.(8), Mauer, G.(9), Gyöngyösi, M.(10)

Medical University of Vienna, Austria, Semmelweis Medical University, Budapest, Hungary

dominika.lukovic@meduniwien.ac.at

Background. Short repeated periods of occlusion/reperfusion after the reopening of the infarct-related artery represent ischemic postconditioning (IPostC). IPostC may induce cardioprotection preventing reperfusion injury. The mechanisms behind the cardioprotective effect of IpostC have been extensively studied, but still little is known. Here, we investigated the effect of IPostC on the gene expression response in ischemic and remote myocardium in porcine closed-chest reperfused acute myocardial infarction (AMI) model utilizing NGS technology.Methods. Domestic pigs underwent induction of AMI by 90-min coronary balloon occlusion of the mid LAD followed by deflation of the balloon. The pigs were randomized to ischemic non-

conditioning (group AMI; n=12) and ischemic post-conditioning (group IpostC; n=12) groups. IpostC was performed immediately after initiation of reperfusion by repeated 3x5 min. coronary balloon inflation/deflation. Sham-operated pigs served as control (n=6). Hearts were explanted after 3 hours (n=6 of each group) and 3 days (n=6 of each group) post/sham-AMI and transcriptomic analysis was performed.Results. Gene ontology enrichment analysis showed significant overexpression of genes involved in focal adhesions in IPostC group as compared to AMI group. Upregulation of key receptors of focal adhesion signalling pathway was pronounced in IPostC group in remote and infarction area at 3 days post-AMI. This led to further stimulation of PI3K/Akt survival signalling pathway. PTEN, PRKCA and PIK3 were overexpressed also in AMI group at 3 days post occlusion, whereas 3 hours post-occlusion animals showed non-significant regulation. Conclusion. We demonstrate first in a translational porcine model of iPostC that stimulation of focal adhesion signalling pathway leads to upregulation of cytoskeletal-based survival signalling. This may play a prominent role in protecting the myocardium from ischemia-reperfusion injury.

194 Arterial hypertension is associated with the DNase I single nucleotide polymorphism Q222R and enhanced neutrophil extracellular trap formation

Müller, J. (1), Hofbauer, T. (2), Scherz, T. (3), Panzenböck, H. (4), Staier, N. (5), Heidari, H. (6), Dujmic, J. (7), Mangold, A. (8), Lang, I.M. (9)

Medical University of Vienna

n1142556@students.meduniwien.ac.at

BACKGROUND: DNase I degrades neutrophil extracellular traps (NETs), an important effector mechanism of polymorphonuclear cells (PMNs). The Q222R single nucleotide polymorphism (SNP) in the DNase I gene impairs its function significantly. This SNP is associated with a higher incidence of myocardial infarction. In a model of spontaneously hypertensive rats, DNase I activity is decreased. We hypothesized that 1) Q222R is associated with hypertension and decreased NET degradation and 2) that high blood pressure (RR) leads to increased NET formation.METHODS: DNA (n=274, male=77%, age=59±13y) and PMNs (n=18, male=78%, age=64±10y) were isolated from STEMI patients at the Medical University of Vienna. Q222R SNP (ID rs 105384) was analyzed by reaction restriction fragment length PCR. PMNs were stimulated with 2.5µM phorbol-12-myristate-13-acetate (PMA) and NET formation was measured using a fluorescence reader-based quantification assay.RESULTS: Hypertension was more common in patients with a homozygous Q222R SNP (84% vs. 62%, p=0.046). In patients with hypertension, both systolic and diastolic RR were positively correlated with NET formation after stimulation with PMA (systolic r=0.479, p=0.044; diastolic r=0.600, p=0.008).CONCLUSION: Impaired DNase I activity leads to increased levels of extracellular DNA, a danger-associated molecular pattern (DAMP) which leads to chronic inflammation. This

results in higher systemic blood pressure, which also fosters the proinflammatory milieu by ROS-mediated activation of PMNs.

195 Inducing Angiogenesis with a Trans-coronary Sinus Catheter Intervention in an Ischemia/Reperfusion Model of Porcine Hearts

Nebert, C (1) Aschacher, T. (1), Eichmair, E. (1), Messner, B. (1), Mohl, W. (1)

Medical University of Vienna

clemens.nebert@meduniwien.ac.at

BackgroundAs we know from prior studies manipulating cardio venous endothelium with a trans-coronary sinus catheter intervention opens pathways for endogenous repair.Therefore, we assessed the hypothesis that a coronary sinus catheter intervention substantiates angiogenesis and initiates cardiac repair in an ischemia/reperfusion model.Material and Methods32 open chest pigs were divided: sham-operation (n=3); 4 hours Infarct and 1 hour reperfusion (control-group, n=8), 4 hours PICSO without infarct and reperfusion (PICSO-A, n=10); PICSO with infarct and reperfusion (PICSO started 15 min. after ischemia (PICSO-B, n=11). Specimen were taken from: LAD region (infarct), adjacent zones Border1 and 2, Circumflex region remote R, Right ventricle RV. VEGFR1, 2 positive arteries and veins were calculated as percentage of total number of vessels, p53 positivity was measured as percentage of total amount of pixels and Ki67 expression was calculated as total number of cells using confocal-microscopy.ResultsVEGFR1 was significantly upregulated in arteries and veins of both interventional groups as compared to the control ($p<0.05$). VEGFR2 expression in arteries of PICSO B and in veins of both PICSO groups was significantly higher than in the control group ($p<0.05$). p53 was significantly downregulated in myocardial tissue of PICSO A animals as compared to the control animals ($p<0.05$). Ki67 was significantly higher expressed in PICSO A as compared to the control ($p<0.05$).ConclusionIn conclusion, this study affirms the theory that a simple manipulation of the coronary sinus with a catheter intervention has beneficial effects on myocardial jeopardy by stimulating neoangiogenetic and cardioprotective mechanisms.

196 Argon Preconditioning Protects Human Adult Cardiomyocytes from Oxygen-Glucose-Deprivation Induced Injury

Soto Gonzalez,L.(1) Qi H. (2), Hafner C.(3), Kaun C.(4), Krychtiuk K.(5), Wojta J.(6), Tretter V.(7), Markstaller K.(8), Klein K.U.(9)

Department of Anesthesia, General Intensive Care and Pain Management, Medical University of Vienna, Austria. Department of Internal Medicine II, Medical University of Vienna, Austria. Core Facilities, Medical University of Vienna, Austria. Ludwig-Bolzmann Cluster for Cardiovascular Research, Austria.

lourdes.soto-gonzalez@meduniwien.ac.at

Objectives: The noble gas argon is a potential cost-effective alternative to xenon with regard to myocardial preconditioning before ischemia-reperfusion injury. Aim of this in-vitro study was to investigate the dose-dependent effects of argon preconditioning on human adult cardiomyocytes (HAMCs) under oxygen-glucose deprivation.Methods: With approval of the local ethics committee and after obtaining written patient informed consent, HACMs were isolated from explanted hearts of transplantation patients. Passage 3 cells were exposed to 30 and 50% argon for 90 minutes and 16h of oxygen-glucose deprivation. All analyses were performed in triplicates and all experiments were performed three times. At 0h and 16h the different states of apoptosis were quantified by flow cytometry and analyses were performed.Results: Oxygen-glucose deprivation resulted in a 30% decrease of viable cells. Preconditioning with argon 30 or 50% restored full vitality as seen in the control group. Pathway specific inhibitors mitigated the protective effect of argon 30 and 50%. These results were confirmed by TUNEL staining analysis. Argon preconditioning induced a reduction of IL-6, IL-8 and LDH secretion during oxygen-glucose deprivation. We could show an activation of p-ERK and p-JNK during argon exposure by Westernblot analysis. Conclusion: Preconditioning with 30% or 50% argon is cell protective with regard to subsequent oxygen-glucose deprivation. We could show, that the protective mechanism included an activation of MAP kinases and Akt. As argon is cost-effective, generally available and easy to administered, pre- and perconditioning therapy using argon might be an interesting novel cardioprotective option.

197 A flow model to monitor endothelial activation induced by septic plasma and to assess the effect of mediator modulation

Eichhorn, T. (1)*, Rauscher, S. (2), Hammer, C. (1,2), Gröger, M. (2), Fischer, M.B. (1,3), Weber, V. (1,3)

(1) Christian Doppler Laboratory for Innovative Therapy Approaches in Sepsis, Danube University Krems, Austria (2) Core Facility Imaging, Medical University of Vienna, Austria (3) Center for Biomedical Technology, Danube University Krems, Austria

tanja.eichhorn@donau-uni.ac.at

Endothelial activation is a key pathomechanism of sepsis and is associated with excessive recruitment and adhesion of leukocytes. Treatment of sepsis patients represents a major challenge due to their extreme heterogeneity. Extracorporeal mediator modulation has been suggested as an adjunctive therapy to reduce endothelial activation. The aim of this study was to establish a flow model to monitor endothelial activation induced by lipopolysaccharide (LPS)-stimulated whole blood or by septic blood and to assess the effect of cytokine adsorption on endothelial activation. Conditioned medium was prepared by diluting plasma tenfold with cell culture medium and was used to stimulate human umbilical vein endothelial cells (HUVEC) under static conditions or under flow. The effect of cytokine adsorption on HUVEC activation was studied by pre-treating LPS-stimulated whole blood or septic plasma with polystyrene divinylbenzene-based adsorbents. Conditioned medium from LPS-stimulated blood induced HUVEC activation, as indicated by increased release of interleukin (IL)-6, IL-8, plasminogen activator inhibitor-1, upregulated expression of intercellular adhesion molecule-1 and E-selectin, as well as by enhanced adhesion of monocytic THP-1 cells. Pre-treatment with adsorbents efficiently reduced cytokine levels and attenuated endothelial activation. Plasma samples from sepsis patients containing comparable IL-6 and TNF-alpha levels substantially differed regarding their potential to induce HUVEC activation. Monocyte adhesion correlated with elevated levels of granulocyte-colony stimulating factor and monocyte chemotactic protein-1, while IL-1 receptor antagonist, IL-10, and interferon-inducible protein-10 were elevated in plasma samples that failed to induce monocyte adhesion. In conclusion, septic plasma samples showed profound differences in their potential to trigger endothelial activation and cytokine modulation strongly reduced endothelial activation and monocyte adhesion.

198 CXCL5 alters metastatic patterns of malignant melanoma

Forsthuber, A. (1) Forsthuber, A. (1)

(1) Skin and Endothelium Research Division (SERD), Abteilung für Allgemeine Dermatologie und Dermatoonkologie, Medizinische Universität Wien; (2) Division of General Dermatology and Dermatooncology, Department of Dermatology, Medical University of Vienna, Austria; (3) Core Facility Imaging, Medical University of Vienna, Austria

agnes.forsthuber@meduniwien.ac.at

Previous analysis of human and mouse melanoma chemokine profiles showed that high expression of CXCL5 is in accordance with a worse disease progression in terms of lymph node metastasis. To investigate the role of CXCL5, an immune competent melanoma C57BL/6 mouse model, using CXCL5/LIX overexpressing B16F1 cells, was established. CXCL5 expressing melanoma strongly recruited neutrophils to the primary tumor and showed higher frequencies of lymph node metastasis than the wt control tumors. Additionally, metastasis of CXCL5 expressing tumors was restricted to a lymphogenic route, whereas the wt control tumors

metastasized via lymphatic vessels as well as blood vessels. Chemokine profiling of CXCL5 overexpressing tumors versus control shows that changing the expression of one single chemokine does not affect the expression pattern of other well-known pro tumorigenic chemokines. This gives CXCL5 and its recruited neutrophils more importance being the active key players in melanoma lymph node metastasis. In vivo experiments using a neutrophil depletion antibody and a CXCL5 neutralizing antibody will unravel the specific effects of neutrophils and CXCL5 separately on disease progression. Additionally, samples from human melanoma xenografted in SCID mice as well as melanoma patient samples will be analysed for the presence of CXCL5 and correlated to disease outcome.

199 Prevention of Neointimal Hyperplasia in Vascular Grafts

Finsterwalder, R. (1) Ganesan, MK. (1), Leb, H. (1), Habertheuer, A. (2, 3), Wiedemann, D. (2), Petzelbauer, P. (1)

(1) Skin & Endothelium Research Division, Department of Dermatology, Medical University of Vienna, (2) Department of Cardiac Surgery, Medical University of Vienna, (3) Hospital of the University of Pennsylvania, Division of Cardiovascular Surgery

richard.finsterwalder@meduniwien.ac.at

A vast number of patients is affected by blood vessel occlusion which can be fatal if left untreated. One treatment option for blood vessel stenosis is bypass grafting. The aim of this technique is to circumvent a stenosed area by transplanting a healthy vessel to ensure sufficient blood supply. A drawback of this approach is the relatively poor long term performance of the grafted tissue due to vascular occlusion for which neointimal hyperplasia is one of the major predisposing risk factors.A healthy blood vessel consists of an endothelial cell monolayer which is in constant contact with the circulating blood forming a highly selective barrier between underlying tissue and circulation. The underlying elastic membrane (i.e. tunica intima) separates the endothelium from multiple layers of smooth muscle cells providing mechanical strength to the blood vessel.The main feature of neointimal hyperplasia is an increase in smooth muscle cell number combined with a breakdown of the intima which ultimately leads to a decrease in blood vessel lumen. In addition to this, an increase of extracellular matrix proteins can be observed as well as an accumulation of immune cells. These changes ultimately lead to restenosis and occlusion of the graft.The aim of this study is to elucidate the molecular mechanisms which trigger neointimal hyperplasia. Therefore, a well-established and widely used mouse model is applied in which a donor vessel is interposed into the carotid artery of a recipient mouse. In order to characterize events, which take place immediately during and after grafting RNA sequencing was performed after 1h, 6h and 24h of re-perfusion. Following this, the identified events are further investigated in order to (1) characterize initial events after surgery and (2) to therapeutically interfere with these processes in order to reduce the risk of restenosis.

200 Characterization of left ventricle function in a relevant experimental model for human rheumatoid arthritis

Goncalves, I.F.(1), Hayer, S.(2), Inci, M.(1), Niederreiter, B. (2), Shvets, T.(2), Redlich, K.(2), Kiss, A.(1), Podesser, B. K.(1)

Medizinische Universität Wien

ines.fonsecagoncalves@meuniwien.ac.at

Background: Rheumatoid arthritis (RA) is associated with left ventricle (LV) hemodynamic dysfunction which may be due to the upregulation of the circulatory levels of cytokines and chemokines. However, their expression and contribution to LV dysfunction in RA heart are unknown. The activation of neuregulin-1 (NRG1)/receptor Tyrosine Kinase 2 and 4 (Erbb2 and Erbb4) pathway is considered to protect the myocardium and the impairment of this pathway, ultimately contributes to the development of heart failure. Aim: Characterize LV function and determine the expression of inflammatory cytokines, chemokines, NRG1/ErbB in hearts of a TNF-driven inflammatory, erosive arthritis model. Methods: Anaesthetized and intubated male and female 14-15 weeks old human TNF-alpha transgenic (hTNFg; n=7) and their wild type (Wt) littermates mice (n=7) were used. LV function was evaluated by inserting the catheter tip retrograde into LV. mRNA levels of cytokines and chemokines (IL-6, IL-1β, MCP-1, MIP2 and KC), NRG1, Erbb2 and Erbb4 in LV samples were determined by RT-qPCR. Results: hTNFg mice showed severe arthritis such as paw swelling in association with smaller body and heart weight in comparison to Wt littermates ($P<0.01$, respectively). LV systolic pressure and the rate of LV pressure rise was decreased in hTNFg mice ($P<0.01$, respectively). In comparison with Wt littermates mRNA expression of MCP-1 and KC were increased in hTNFg mice ($P<0.01$, respectively). NRG1 expression gradually increased in hTNFg mice compared to Wt littermates. Female mice showed a downregulation of ErbB2 expression, irrespectively of RA. Conclusion: hTNFg mice have showed impaired LV systolic function in association with the upregulation of MCP-1 and KC. NRG1 expression increased in both gender and downregulation of ErbB2 was observed in female mice, irrespectively of RA. These results may represent a potential novel therapeutic point to improve LV function and reduce risk of cardiovascular disease in RA.

201 Impact of endoplasmic reticulum stress on melanoma malignancy

Eigner, K. (1), Filik, Y. (1), Klambauer, G. (2), Swoboda, A.(3), Moriggl, R. (3), Stangl, H.(1), Mikula, M.(4), Röhrl, C.(1)

(1) Department of Medical Chemistry, Center for Pathobiochemistry and Genetics, Medical University of Vienna, (2) Institute of Bioinformatics, Johannes Kepler University Linz, (3) Ludwig Boltzmann Institute Cancer Research, Vienna, (4) Department of Medical Genetics, Center for Pathobiochemistry and Genetics, Medical University of Vienna

karin.eigner@meduniwien.ac.at

Melanoma is the most aggressive type of skin cancer and patients with metastatic melanoma display a five years overall survival rate of less than 5%, whereas before metastasis, survival rate is 99%. So far, chemotherapy and anti-cancer drugs did not achieve the desired results in the treatment of melanoma and most patients acquire resistance during therapy. In the last few years endoplasmic reticulum stress (ER stress) and the unfolded protein response (UPR) gained attention, due to its abnormal regulation in various cancer types. ER stress on the one hand enables adaption to altered metabolic demands and on the other hand triggers apoptosis. In this project we found that ER stress triggers melanoma malignancy in vitro.In isogenic cell lines we show that ER stress is increased in metastatic melanoma cells. Analyzing the UPR we found activation of the ATF6 and PERK pathways, but not the IRE1 pathway. Available data from the literature confirmed that the ATF6 and PERK branches of the UPR are up-regulated in human melanoma patients and associated with poor survival. Moreover, ER stress was also detected in lymph nodes in a melanoma mouse model. Array data and gene set enrichment analysis revealed increased expression of genes involved in migration, invasion and growth factors in metastatic melanoma cells and ER stress dependence. In functional assays 4-PBA was used to ameliorate ER stress and indeed, invasion and migration of metastatic melanoma cells was reduced.Taken together, we show that ER stress triggers melanoma malignancy by up-regulating genes involved in migration, invasion and growth factors in vitro. Thus, the investigation of ER stress and the UPR in melanoma seem to be a promising target in cancer therapy.

202 Differential in vivo activation of monocyte subsets during experimental endotoxemia in humans

Thaler, B (1), Hohensinner PJ(1), Krychtiuk K.A.(1,2), Koller L(1), Brekalo M(1), Maurer G(1), Huber K(3), Matzneller P(4), Zeitlinger M(4), Jilma B(4), Wojta J(1,2,5), Speidl WS(1)

(1) Department of Internal Medicine II, Medical University of Vienna, Vienna, (2) Ludwig Boltzmann Cluster for Cardiovascular Research, Vienna, (3) 3rd Medical Department for Cardiology and Emergency Medicine, Wilhelminen Hospital, Vienna, (4) Department of Clinical Pharmacology, Medical University of Vienna, Vienna, (5) Core Facilities, Medical University of Vienna, Vienna, Austria

barbara.thaler@meduniwien.ac.at

BACKGROUND: Human monocytes are a heterogeneous cell population that can be divided into a classical (CM, CD14++CD16-), a non-classical (NCM, CD14+CD16+), and an intermediate subset (IM, CD14++CD16+). Monocytes are key cells in the response to sepsis. A human endotoxemia model was used to identify monocyte subset activation under septic conditions.METHODS: Healthy volunteers (n=12) were injected with a bolus infusion of LPS (2ng/kg) and blood samples were obtained before LPS injection and at 2h, 6h and 24h after injection. Whole blood samples were stained for CD14, CD16 and CD11b and were analysed with a BD FACS Canto II. Absolute cell numbers were determined using 123counting beads and a novel in situ mRNA hybridization approach to detect IL6 and IL8 specific mRNA at the single-cell level by flow cytometry was appliedRESULTS: The analysis of cell counts showed a drop in monocyte levels after 2h of LPS treatment. After 6h, CM recovered to their initial cell number whereas IM and NCM remained reduced. After 24h, monocyte subsets were skewed towards IM, which showed a 572% increase ($p<0.001$). In addition, IM showed the strongest upregulation of CD11b after 2h compared to CM and NCM ($p<0.05$, $p<0.005$). After 6h and 24h CD11b returned to baseline values albeit IM still displayed the highest baseline expression. Furthermore, IL6 and Il8 mRNA levels were enhanced after 6h in IM to 180% ($p<0.05$) and 240% ($p<0.05$) respectively and NCM to 225% ($p<0.05$) and 232% ($p<0.05$) respectively, whereas CM response was weak to 119% ($p<0.05$) and 142% ($p<0.05$) respectively. After 24h, IM and NCM mRNA levels for IL6 and IL8 mRNA return back to the baseline expression levels. CONCLUSION: These results show that monocyte subsets are activated differently during endotoxemia in vivo. Especially CD16 positive cells react strongly to LPS treatment resulting in higher CD11b, IL6 and IL8 levels compared to CD16 negative cells.

203 The Platelet Proteome - Changes through ex-vivo influences

Schmidt, G. (1), Reumiller, C. (2), Hellinger, R. (3), Jilma, B. (4) & Zellner, M. (5)

Medical University of Vienna

georg.schmidt@meduniwien.ac.at

BACKGROUND: Obtaining valid data that reflect in-vivo platelet status and proteome as accurate as possible is crucial in platelet studies. After leaving the vascular system, however, platelets are exposed to ex-vivo influences that can distort the platelets´ in vivo phenotype and function. The first and possible strongest ex-vivo influence is the indispensable inhibition of the blood coagulation and platelet activation. This study therefore wants to elucidate possible differences in platelet function and platelet proteome that are induced by different ways of inhibiting the coagulation system and platelet activation. METHODS: We therefore took blood from six healthy volunteers both in Sodium-Citrate blood tubes (0.11mM citrate) and CTAD tubes - a combination of anticoagulant (0.11mM citrate) and antiplatelet-drugs (theophylline, adenosine and dipyridamole) - and analysed the platelet proteome by two-dimensional

differential gel-electrophoresis (2D-DIGE). RESULTS: We could observe five specific and strong protein abundance changes between Citrate and CTAD ranging from 1.9 x to 5.6 x fold change (adjusted p-values < 0.05). Similar modifications in the platelet protein profile were induced by the addition of prostaglandin I[down2] (PGI[down2]) to citrated blood. These abundance changes could not be induced by ADP activation (0.5[my]M). Identified spots are mainly involved in the cytoskeletal organization (LASP1, Zyxin, and Caldesmon) and integrin activation (RAP1b, SKAP2). DISCUSSION: The majority of platelet proteins was unchanged if blood was supplemented with CTAD or PGI[down2], except for a few distinctively changed protein species related to cytoskeletal activity and integrin activation. Our results indicate a platelet inactivation proteome whose abundance changes are probably based on post translational modifications (PTMs) induced by both antiplatelet drugs and PGI[down2] via the cAMP/Proteinkinase A pathway.

204 Validation of a luminescence-based NET degradation assay

Hofbauer, T., Mangold, A., Scherz, T., Staier, N., Müller, J., Heidari, H., Dujmic, J., Lang, IM.

Medical University of Vienna

thomas.hofbauer@meduniwien.ac.at

BACKGROUND: Formation of neutrophil extracellular traps (NETs), an effector mechanism of polymorphonuclear cells (PMNs), promotes thrombosis. Degradation of NETs is predominantly mediated via DNase 1. Impaired NET degradation is related to cardiovascular events and increased myocardial infarct size. Mechanisms influencing NET degradation are poorly understood. It is known that serine proteases like thrombin, plasmin and heparin support DNase 1 activity. We aimed to validate a luminescence-based NET degradation assay (NDA) to investigate mediators of NET degradation.METHODS: PMNs were stimulated with PMA to generate NETs in 48-well culture plates. Media containing DNase 1 and respective reagents were added. After degradation of NETs and release of double-stranded DNA (dsDNA) fragments supernatants from each well were transferred to a 96-well plate. PicoGreen (Thermo Fisher Scientific, P11496) was added to the wells. Lambda DNA served as a positive control and dsDNA levels were measured on a luminescence reader.RESULTS: In the presence of thrombin, NET degradation by DNase 1 was significantly enhanced in a dose-dependent manner (see figure), in the absence of serine proteases or heparin. Plasmin and heparin alone promoted NET degradation. EDTA completely inhibited DNase 1 activity.CONCLUSION: The NDA is an efficient assay to evaluate the influence of any co-factor for DNase 1 activity and facilitates the analysis of the complex interplay between NET formation and degradation

205 Influence of KDR Knock out on Pulmonary Vascular Disease

Theresa-Marie Dachs(1)Max-Paul Winter (1), Arman Alimohammadi(1), Adelheid Panzenboeck(1), Johanna Altmann (1), Smriti Sharma (1), David Santer (2), Felix Nagel (2), Bruno Podesser (2), Maria Sibilia (3), Irene Lang (1)

(1)Department of Cardiology, Internal Medicine II, Medical University of Vienna , (2) Institute of Biomedical Research, Medical University Vienna, (3) Comprehensive Cancer Center Medical University of Vienna

n1242513@students.meduniwien.ac.at

BackgroundPulmonary arterial hypertension (PAH) is a severe progressive disease characterized by obstruction of small pulmonary arteries leading to increased pulmonary vascular resistance. The key pathologic finding is a negative pulmonary vascular remodeling process with total vessel occlusion and a monoclonal expansion of collateral endothelial cells. Aim of our study was to investigate if inhibition of VEGFR-2 (KDR) by direct gene manipulation may replicate classical pulmonary vasculopathy.MethodsWe utilized mice with conditional VEGFR-2/KDR knock-out in endothelial cells (KDR-/-). KDRflox/flox/Tie-2Cre and KDRflox/flox/Tie-2 mice were injected intraperitoneally with tamoxifen for three weeks to induce the knock-out. KDR-/- mice and wild type littermates were held in an environmental chamber with FiO2 of 10% or under normoxia for 2, 4, and 6 weeks. We investigated the effect of KDR deletion and chronic normobaric hypoxia on pulmonary hemodynamics and right ventricular hypertrophy.ResultsAfter KDR knockout mice revealed a significant increase in VEGF and BNP serum levels (Fig.1). RT-PCR indicated a significant downregulation of the BNP pathway as consequence of KDR knockout (Fig.2). KDR-/- mice showed significantly increased right ventricular pressures (RVSP's) and Fulton indices after normoxic and hypoxic conditions, compared with wild type controls (Fig.3), whereas there was no significant difference in systemic arterial pressure. Knockout mice showed a significant increase in pulmonary arterial wall thickness and significant increased α-SMC positive area measured by tissue FACS. We observed the loss of isolectin-4 positive microvessels in the knockout group. Most interestingly lung histologies demonstrated neointimal thickening and vessel occlusions in lungs of KDR-/- mice resembling human pulmonary arteriopathy.ConclusionClassical pulmonary arterial hypertension was induced in C57/BL6J mice by direct ablative gene manipulation of KDR.

206 APOSEC ameliorates wound healing in genetically diabetic db/db mice

Traxler, D (1), Keibl, C. (2), Lidinsky, D. (2), Marsh, L.M. (3), Nuernberger, S. (2), Hartinger, J. (2), Redl, H. (2), Olschewski, A. (3,4), Gabriel, C. (2,5), Ankersmit, H.J. (1,6), Slezak, P. (2)

(1) Christian Doppler Laboratory for Diagnosis and Regeneration of Cardiac and Thoracic Diseases, Medical University of Vienna, (2) Ludwig Boltzmann Institute for Experimental and

Clinical Traumatology, Vienna, (3) Ludwig Boltzmann Institute for Lung Vascular Research, Graz, (4), Department of Experimental Anaesthesiology, Medical University of Graz, (5) Red Cross Blood Transfusion Service of Upper Austria, Linz, (6) Division of Thoracic Surgery, Department of Surgery, Medical University o

denise.traxler-weidenauer@meduniwien.ac.at

Diabetic foot ulcer displays a severe complication of diabetes, however, no sufficient therapeutic option exists. APOSEC, the supernatant of apoptotic PBMCs, has already displayed beneficial effects in tissue regeneration and wound healing in several tissues and disease entities, but has not been tested in a model of diabetic wound healing.We used a common full-thickness wound healing model in genetically diabetic db/db mice. APOSEC or control were topically administered to the wound site for ten consecutive days. Three concentrations of APOSEC were applied. Wound size was measured until day 25 by tracing the wound on acrylic foil and using a stereoscopic camera for 3D wound measurement (circumference, surface area). Masson's trichrome and Weigert's elastic stain as well as immunohistochemical stainings (CD31, CD45) were performed. For additional histological analyses at day 14, further mice were treated with APOSEC or control.We demonstrated that local administration of APOSEC enhances wound healing in genetically diabetic mice. Wound circumference, size of wound surface area and wound size assessed planimetrically were significantly reduced in mice treated with APOSEC at day 18. Additionally a significant dose dependency of the treatment with APOSEC could be shown. Histopathological analyses revealed no differences between APOSEC and control in vessel density, percentage of CD45+ cells, collagen or elastic fibre deposition in the wound zone.We showed that topical application of APOSEC significantly enhances wound healing in diabetic mice and demonstrated also a dose dependent effect, even though histopathological analyses did not exhibit any differences. Chronic diabetic ulcer displays a severe burden. Hitherto no adequate therapy to address this problem exists. PBMCs are a side-product of blood donations and are usually discarded. Utilising the regenerative potential of a yet side-product could provide an inexpensive possibility to address this problem.f Vienna

207 Tenascin-C deficiency attenuates abdominal aortic aneurysm formation

Schaefer, A.K.(1),Nagel, F.(1,2), Kiss, A.(1), Goncalves, I.(1), Kaiser, P.(1), Santer, D.(1,3), Trescher, K.(1,2), Podesser, B.K.(1,2)

(1)Department of Biomedical Research, Medical University of Vienna, (2)Department of Cardiac Surgery, University Hospital of St. Pölten, Austria,(3)Department of Cardiovascular Surgery, Herzzentrum Hietzing, Vienna, Austria

anne-kristin.schaefer@meduniwien.ac.at

Objectives: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 CaCl2 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 before AAA induction and before organ harvesting after 3 and 10 weeks. 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 3 weeks (TNC KO: 1.39±0.25, WT: 1.67±0.22 $p<0.05$) and 10 weeks (TNC KO: 1.51±0.47, WT: 1.98±0.55 $p<0.05$) after AAA induction. No significant changes in diameter ratios were found in sham groups (3 weeks: TNC KO: 0.92±0.08, WT: 0.96±0.22, n.s., 10 weeks: TNC KO: 1.05±0.16, WT: 0.94±0.10, n.s.). Additionally, the score of aortic wall elastin structure disruption was significantly lower in TNC KO than in WT mice 10 weeks after AAA induction (TNC KO: 3.32±1.15, WT: 4.25±0.75 $p<0.05$).Conclusions:In our study we found first evidence that TNC deficiency is associated with reduced AAA formation. To identify possible causal pathways immunohistological and molecular biological assessments will be conducted.

208 Prognostic Relevance of the Pulmonary Artery Diameter in Relation to the Ascending Aorta

Kammerlander, A. (1)Aschauer, S. (2), Zotter-Tufaro, C. (3), Duca, F. (4), Knechtelsdorfer, K (5), Wiesinger, M. (6), Schwaiger, M. (7), Dalos, D. (8), Schneider, M. (9), Marzluf, B. (10), Bonderman, D. (11), Mascherbauer, J. (12)

Medical University of Vienna, Division of Cardiology

andreas.kammerlander@meduniwien.ac.at

Background. The pulmonary artery (PA) diameter and its relation to the ascending aorta (PA:Ao ratio) by cardiovascular magnetic resonance (CMR) or computed tomography (CT) have been identified as non-invasive markers for pulmonary hypertension in heart and lung disease. However, the prognostic value of such measurements is largely unknown. Methods and Results. 650 consecutive patients (47.2% female, mean age 56.1±17.1 years) referred to CMR were prospectively enrolled. Diameters of the great arteries were measured in axial black blood images. Based on previous results, a PA:Ao ratio ≥1.0 was chosen as cut-off for further analysis.

The primary endpoint was defined as a composite of cardiovascular hospitalization and death. 131 (20.2%) patients presented with a PA:Ao ratio ≥1.0. These patients were more frequently female (p=0.010), presented with more atrial fibrillation (p<0.001), more diabetes (p<0.001), worse renal function (p<0.001), higher NT-proBNP levels (p<0.001), larger left (p=0.023) and right ventricles (RV, p=0.002), and worse RV function (p<0.001). Patients were followed for 17.8±12.9 months, during which 110 (16.9%) experienced an event. Kaplan-Meier analysis revealed worse event-free survival rates in patients with a PA:Ao ratio ≥1.0 (log-rank, p<0.001). By multivariable Cox-regression analysis, a PA:Ao ratio ≥1.0 was independently associated with outcome, in addition to age, NT-proBNP serum levels, and RV size.Conclusion. The PA:Ao ratio is an easily measurable parameter by CMR. A ratio ≥1.0 identifies patients at risk, most likely due to elevated pulmonary artery pressures. Based on these results, the PA:Ao ratio should routinely be assessed in CMR scans.

209 Diastolic retrograde flow in the descending aorta by cardiovascular magnetic resonance imaging for the quantification of aortic regurgitation

Kammerlander, A. (1) Wiesinger, M. (2), Duca, F. (3), Aschauer, S. (4), Zotter-Tufaro, C. (5), Schwaiger, M. (6), Badre-Eslam, R. (7), Marzluf, B. (8), Bartko, P. (9), Bonderman, D. (10), Mascherbauer, J. (11)

Medical University of Vienna, Division of Cardiology

andreas.kammerlander@meduniwien.ac.at

Background. Echocardiography is the standard method for quantification of aortic regurgitation (AR). However, accurate estimation of the severity of AR by echo may be challenging due to inherent limitations of applied methods. Cardiovascular magnetic resonance imaging (CMR) has recently been advertised as an accurate method for AR quantification, irrespective of acoustic windows.The present prospective study sought to evaluate the usefulness of CMR for the quantification of AR.Methods and Results. 206 consecutive patients (30% female, 55±21 years old) with varying degrees of AR by echocardiography (83 mild, 52 moderate, and 35 severe, 36 with inconclusive echocardiographic results - “moderate to severe” AR) were invited to undergo CMR within 4 weeks. CMR consisted of standard protocols including phase-contrast velocity-encoded imaging for measurement of regurgitant volume (RegV), and regurgitant fraction (RegF) at the sinutubular junction, and assessment of holodiastolic retrograde flow (HRF) in the descending aorta. Severe AR was defined as the presence of HRF in the descending aorta by CMR.Left ventricular (LV) volumes by CMR significantly increased with increasing AR severity by echo (LV end-diastolic volume/body surface area: mild: 77±24ml/m2, moderate: 96±28ml/m2, “moderate to severe”: 106±43ml/m2, severe: 124±34ml/m2; p<0.001), as did RegF at the sinutubular junction (7±15%, 14±15%, “22±17%, 35±15%; p<0.001).Among the 135 patients with non-severe AR by echo, 11 (8%) had HRF by CMR, indicating severe AR. Among

the 35 patients with severe AR by echo, 12 (34%) did not show HRF by CMR, suggesting overestimation of AR severity in these patients. In patients with inconclusive echo results, 42% had HRF in the descending aorta, indicative for severe AR. Conclusion. Quantification of AR by CMR is feasible and highly reproducible. HRF in the descending aorta by CMR is an easy marker that helps to distinguish between severe and non-severe AR.

210 Accumulation of Cardiac Extracellular Matrix is associated with Adverse Outcome in Patients with Chronic Heart Failure

Duca, F. (1) Zotter-Tufaro, C. (2), Kammerlander, AA. (3). Panzenboeck, H. (4), Boerries, B. (5), Aschauer, S. (6), Agis, H. (7), Kain, R. (8), Mascherbauer, J. (9), Bonderman, D. (10).Medical University of Vienna, Division of Internal Medicine II, Department of Cardiology (1-6, 9, 10),

franz.duca@meduniwien.ac.at

Background. Accumulation of extracellular matrix (ECM) is known to play a crucial role in the pathophysiology of heart failure. However, its prognostic relevance is poorly investigated. Objectives. We aimed to elucidate the influence of ECM area on outcome in various non-ischemic heart failure (HF) types. Methods. A total of 73 HF patients who underwent left ventricular (LV) endomyocardial biopsy (EMB) were enrolled in our study. ECM area was quantified by TissueFAXS and ImageJ software. Patients were followed prospectively in 6-month intervals. The study endpoint was defined as hospitalization for cardiac reason and/or cardiac death. The prognostic relevance of ECM area was tested in multivariable Cox regression analyses. Furthermore, the influence of ECM area on invasively assessed hemodynamic parameters was tested.Results. During a mean follow-up period of 14.0[+-]13.9 months, 34 patients (46.6%) reached the combined endpoint. Median ECM area was 30.5%. Patients with ECM area ≥30.5% experienced significantly more events (67.6% vs. 25.0%, p<0.001) in comparison to patients with ECM area <30.5%. ECM area was independently associated with outcome in the total HF cohort (p<0.001, HR 1.040, 95% CI 1.018-1.062) as well as in HF patients with preserved (HFpEF, p=0.014, HR 1.085, 95% CI 1.017-1.158) and HF patients with reduced ejection fraction (HFrEF, p=0.011, HR 1.144, 95% CI 1.031-1.269). Kaplan-Meier curves and respective Log-Rank tests show a worse event-free survival for patients with ECM area ≥ median in the entire cohort (p<0.001) as well as in the subgroups with HFpEF (p=0.043) and HFrEF (p=0.002).Positive correlations were found between ECM area and pulmonary artery wedge pressure (p=0.042, R=0.249), mean pulmonary arterial pressure (p=0.258, R=0.035), as well as right atrial pressure (p=0.353, R=0.003), whereas stroke volume index (p=0.045, R=-0.247) was inversely correlated with ECM area.Conclusion. ECM area within the LV m

211 Riociguat - new therapeutic approach in the management of cardiac amyloidosis

Duca, F. (1) Zotter-Tufaro, C. (2), Kammerlander, AA. (3). Boerries, B. (4), Aschauer, S. (5), Agis, H. (6), Mascherbauer, J. (7), Bonderman, D. (8).

Medical University of Vienna, Division of Internal Medicine II, Department of Cardiology (1-5, 7, 8), Division of Oncology, Department of Internal Medicine I, Medical University of Vienna, Vienna, Austria (6)

franz.duca@meduniwien.ac.at

Background:Cardiac amyloidois (CA) is a rare disease and represents the prototype of a restrictive cardiomyopathy. A vast majority of affected patients present with advanced heart failure and face significant morbidity and mortality. However, an effective therapy is still lacking and a diagnosis of CA precludes patients from participation in standard heart failure clinical trials. We aimed to test the safety and efficacy of Riociguat in patients with cardiac amyloidosis. Methods:Baseline work-up of patients and re-evaluation under therapy included the assessment of blood pressure, NYHA functional class, exercise capacity as measured by the 6-minute walk test (6MWT), serum NT-proBNP and invasively measured hemodynamic parameters.Results:Six participants with wild-type transthyretin amyloidosis were included in the named-patient use program. Follow-up was performed after a median of 4.5 months (3.0-7.0). Our preliminary findings indicate that application of riociguat in CA patients is safe. Systolic blood pres...

212 Extracellular Volume by Cardiac Magnetic Resonance T1 Mapping is Associated with Outcome in Patients with Heart Failure and Preserved Ejection Fraction

Franz, D. (1), Kammerlander, AA. (2) Zotter-Tufaro, C. (3) Aschauer, S. (4) Schwaiger, M. (5) Marzluf, B. (6) Bonderman, D. (7) Mascherbauer, J. (8)

Medical University of Vienna, Division of Internal Medicine II, Department of Cardiology (2-5, 7,8), the Department of Thoracic Surgery, Otto Wagner Hospital, Vienna (6)

franz.duca@meduniwien.ac.at

BackgroundMyocardial extracellular volume (ECV) accumulation is one of the key pathophysiologic features of heart failure with preserved ejection fraction (HFpEF). Our aims were to 1. measure ECV by cardiac magnetic resonance (CMR) T1 mapping using the modified Look-Locker inversion recovery (MOLLI) sequence; 2. validate MOLLI-ECV against histology; and 3. investigate the relationship between MOLLI-ECV and prognosis in HFpEF. Methods117 consecutive HFpEF patients underwent CMR, coronary angiography, and invasive hemodynamic

assessments at baseline. 18 patients also underwent left ventricular biopsy for histological analysis (Histo-ECV). To assess the prognostic impact of MOLLI-ECV, its association with hospitalization for cardiovascular reasons / cardiac death was tested by multivariable Cox regression analysis.ResultsHisto-ECV was 30.1 [+-] 4.6% and was significantly correlated with MOLLI-ECV (R= 0.494, p=0.037). Patients were followed for 20.4 [+-] 13.3 months, during which 34 had a cardiac event. By Kaplan-Meier analysis, patients with MOLLI-ECV ≥ the median (28.9%) were at greater risk of cardiac events (log-rank, p=0.028). MOLLI-ECV significantly correlated with NT-proBNP (p<0.001), 6-minute walk distance (p=0.004), NYHA functional class (p=0.009), right atrial pressure (p=0.037), heart rate (p=0.039), and stroke volume (p=0.043). By multivariate Cox regression analysis MOLLI-ECV was independently associated with outcome among imaging variables (p=0.038) but not after adjustment for clinical and invasive hemodynamic parameters. ConclusionsWe demonstrate that MOLLI-ECV in HFpEF accurately reflects histological ECV, correlates with markers of disease severity, and is independently associated with outcome among imaging parameters. MOLLI-ECV has the potential of becoming an important biomarker in HFpEF.

213 Histological Validation of ECV Quantification by Cardiac Magnetic Resonance T1 Mapping in Cardiac Amyloidosis

Franz, D. (1) Kammerlander, AA. (2) Zotter-Tufaro, C. (3) Aschauer, S. (4) Schwaiger, M. (5) Marzluf, B. (6) Bonderman, D. (7) Mascherbauer, J. (8)

Medical University of Vienna, Division of Internal Medicine II, Department of Cardiology (2-5, 7,8), the Department of Thoracic Surgery, Otto Wagner Hospital, Vienna (6)

franz.duca@meduniwien.ac.at

Background. Cardiac amyloidosis (CA) is caused by accumulation of amyloid fibrils in the myocardium, which leads to an increase in extracellular volume (ECV). Cardiac magnetic resonance (CMR) T1 mapping allows accurate non-invasive ECV measurement. However, it has not been investigated weather CMR-ECV accurately quantifies ECV in CA. Materials and Methods. 21 CA patients were enrolled into our study between July 2011 and November 2015. The study population consisted of 7 (33.3%) wild-type transthyretin (TW-TTR) and 14 (66.6%) light chain (AL) CA patients. All patients underwent myocardial biopsy (EMB) and CMR. EMB specimens were stained with Modified Trichrome and ECV was quantified via ImageJ software using, a color-threshold macro. CMR-ECV was quantified with T1 mapping using the Modified Look-Locker Inversion recovery (MOLLI) sequence. Spearman's correlation and Bland-Altman plots were used for correlation analysis and assessment of agreement between histological ECV (Histo-ECV) and CMR-ECV. Results. All CA patients (N=21) had a CMR-ECV median of 48.5% (23.6%-71.0%), an Histo-ECV median of 50.2% (10.7%-80.6%), a median difference between CMR-ECV and Histo-ECV of -0.9% (limits of agreement -25.9% - 24.1%) and a

correlation coefficient of R=0.752; p<0.001. Conclusions. We are the first to histologically validate CMR-ECV in cardiac amyloidosis patients. Our results show excellent correlation and good agreement of CMR-ECV with Histo-ECV in the whole study population as well as in subgroup analysis for AL-CA and WT-TTR-CA. CMR-ECV quantification by T1 mapping accurately reflects ECV in cardiac amyloidosis patients. As many current phase II and phase III trials are on the way for treatment of CA, ECV measurement via CMR may provide important information on weather CA therapies can indeed reduce the amount of ECV in CA. Therefore, CMR-ECV quantification might be an appealing method in evaluating success and failure of CA therapies.

214 (1) Histological analyses of paclitaxel coated cutting balloon treated in stent restenosis in pigs

Traxler, D (1) Zlabinger, K (1), Spannbauer, A (1), Gyongyosi, M (1)

Division of Cardiology, Medical University of Vienna

denise.traxler-weidenauer@meduniwien.ac.at

Even though with the use of drug-eluting stents (DES) intracoronary stent restenosis (ISR) has become much less common, with the number of stents implanted it still displays a challenge. Reduction in lumen diameter in ISR is the result of neointima formation, which is associated with macrophage accumulation and neovascularisation. Even though treating ISR with DES is a promising treatment option, a drug-eluting balloon (DEB) was considered another attractive therapy. No data on neointima composition in treatment of ISR with DEB exist. The aim of this study was to characterise neointimal tissue in ISR that has been treated with plain cutting balloon (CB) or paclitaxel coated cutting balloon (PCB).Coronary arteries of 8 pigs were stented with bare metal stents (BMS) to induce ISR. After 1 month ISR was treated with either CB or PCB. At 1 month follow-up coronary artery samples were taken for histological analyses. Histomorphometric and histopathological analyses were performed and the presence of CD68+ macrophages was evaluated by immunohistochemical staining.Quantitative coronary angiography showed an equal ISR in both groups one month after BMS implantation.
i;ameter stenosis and minimal lumen diameter were equal after BMS implantation in both groups before treatment. Histomorphometry exhibited a significantly larger lumen area and smaller neointima area in the PCB group. Histopathological analyses revealed significantly less fibrin deposition and adventitial inflammation scores even though injury scores were equal. CD68+ macrophages have been found in 43% of all coronary artery samples, equally distributed in both groups.This study highlights the successful treatment of ISR with PCB and showed reduced formation of neointimal tissue, fibrin deposition and adventitial inflammation in the DEB group. Moreover, it describes the presence of CD68+ macrophages at a similar proportion within neointimal tissue in both groups.

215 APOSEC influcences expression of IL-6 and IL18 in hypoxic porcine cardiomyocytes.

Katrin Zlabinger (1) Romana Michenthaler(2), Michelle Leitgeb(3), Dominika Lukovic(4), Alfred Gugerell(5), Johannes Winkler(6), Ljubica Madic(7), Hendrik J. Ankersmit(8), Mariann Gyöngyösi(9)

Medical University Vienna

katrin.zlabinger@meduniwien.ac.at

Background. We previously described the successful establishment of a porcine cardiomyocyte cell culture model. In this study we further investigated the cell behaviour after induction of hypoxia and analyzed the impacts of a paracrine factor APOSEC (secretom of apoptotic peripheral blood mononuclear cells) on cell morphology and factors IL-6 and IL-18, which are known to be up regulated due to hypoxic stress. Methods. 5x10[up4] porcine cardiomyocytes were seeded into ibidi slides (µ-slide 4 well) and hypoxia was induced adding cobalt (II) chloride hexahydrate (CoCl[down2]) in different concentrations (50-800 µM) for two hours to the cells. Thereafter APOSEC was added after different time points (0h, 2h, 24h) in different concentrations (1:2, 1:4, 1:8, 1:10) to the cells and cell morphology was observed via live cell imaging on Olympus fluorescense microscope. RNA was isolated from cell lysate and the expression of the proinflammatory cytokines IL-6 and IL-18 were measured after 24h or 48h of APOSEC treatment. Results. Based on the changes in cell morphology, 100µM CoCl[down2] per reaction was chosen to be the optimal concentration for hypoxia induction. Hypoxia induction led to an increase of gene expression of IL-6 (by 1.95 fold) and IL-18 (by 1.32-fold) compared to normoxic cardiomyocytes. IL-6 expression of APOSEC treated cells was downregulated in all samples compared to hypoxic cells. IL-18 expression was also down regulated in all of the APOSEC samples except in the samples in which APOSEC was applied 24 hours after hypoxia induction, where IL-18 was extremely up regulated.Discussion. We successfully established a CoCl[down2]-induced hypoxia cell culture model, which was confirmed by up-regulation of IL-6 and IL-18 on RNA level. Furthermore we could observe a positive impact regarding cell morphology and down regulation of IL-6 and IL-18 in cells treated for 24h with APOSEC which was added 2 hours after hypoxia.

216 Measuring the „NET effect“ – establishment of a NET quantification assay

Scherz, T. (1)Hofbauer, T.(1), Mangold, A.(1), Staier, N.(1), Müller, J.(1), Heidari, H.(1), Dujmic, J.(1), Lang, IM.(1)

(1) Medical University of Vienna

thomas.scherz@meduniwien.ac.at

217 COMBINED ORAL ADMINISTRATION OF L-ARGININE AND TETRAHYDROBIOPTERIN IN A RAT MODEL OF PULMONARY ARTERIAL HYPERTENSION

Schreiber, C (1),MS EILENBERG(1), A PANZENBOECK(1), MP WINTER(1), H BERGMEISTER(2), J MASCHERBAUER(1), IM LANG(1), D BONDERMAN(1)

(1) Medical University Vienna, Clinic for Internal Medicine II Dpt. for Cardiology;(2) Medical University Vienna, Institute of Biomedical Research

n0642561@students.meduniwien.ac.at

218 Cell-type specific proteolytic processing of endogenous and exogenous thrombospondin-1

Seif K. (1)Alidzanovic L. (2), Starlinger P. (3), Zommer A. (4), Brostjan C. (5)

Medical University Vienna

katharina.seif@meduniwien.ac.at

219 Characterizing CD40 signaling in platelet-endothelium cell interaction in comparison to TNF signaling

Jacqueline SeignerUlrike Resch, Rainer deMartin

Medical University of Vienna

seigner.jacqueline@gmx.at

220 Neutrophil activation markers are sensitive diagnostic indicators of the abdominal aortic aneurysms

Zagrapan, B. (1) Eilenberg, W. (1), Hetzer, S. (1), Münch, K. (1), Elleder, V. (1), Lackner, E. (2), Drucker, Ch. (2), Jilma, B. (2), Neumayer, Ch. (1), Huk, I. (1), Brostjan, Ch. (1)

(1) Department of Surgery, Medical University of Vienna, Vienna General Hospital, Vienna, Austria; (2) Department of Clinical Pharmacology, Medical University of Vienna, Vienna General Hospital, Vienna, Austria

branislav.zagrapan@meduniwien.ac.at

220A Chronic TNFα exposure leads to premature endothelial senescence via ROS generation and NF-kB activation

Shafaat Yar Khan, Ezzat M Awad, Andre Oszwald, Pavel Uhrin and Johannes M Breuss*

Department of Vascular Biology and Thrombosis Research Medical University of Vienna, Austria

shafaat.khan@meduniwien.ac.at

Replicative senescence (Sc) is a phenomenon describing the cease of cell division. Premature cellular Sc can be induced by environmental stress like oxidative stress and inflammation which contribute, *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). There is scarcity of studies about TNFα induced Sc and its mechanism in endothelial cells (ECs). This study aims to assess the possible role of chronic exposure of TNFα in the induction of premature Sc in ECs with the involvement of NF-kB and reactive oxygen species (ROS) and therefore to prevent it by their modulation by NF-kB inhibitors and antioxidant. We exposed HUVECs to TNFα for a period of 6 days followed by 3 days without stress. We assessed its effects on cell proliferation (cell count &Ki67), cell cycle inhibitors (p16, p21), E-selectin and ICAM-1 expression, ROS production, PAI 1, IGFBP 5 and NF-kB translocation. For the inhibition of NF-kB pathway we used PHA-408 or Plumericin and N-acetylcysteine (NAC) as antioxidant. Chronic exposure of TNFα, led to a substantial decrease in cell proliferation and to an increase in the %age of p16 and p21 positive cells, indicating a successful induction of SC in ECs. SASP induction was evident by increased levels of E-selectin, ICAM-1 expression, PAI 1 and IGFBP 5. Chronic TNFα exposure led to an increase in ROS production and nuclear mean integrated intensity of NF-kB. Inhibition of the NF-kB pathway and use of antioxidant NAC prevented the increase in the %age of ECs positive for p16 or p21, E-selectin, ICAM-1 expression, IGBP5, and ROS production along with prevention in the loss of cell proliferation. Our data provide evidence for a role of ROS and NF-kB signaling to the TNFα-induced development of endothelial Sc and the SASP.