MEDIZINISCHE UNIVERSITÄT
INNSBRUCK

Programme and Abstract book

1st MUI-START Symposium

MUI-START SYMPOSIUM

15 June 2012, Lecture Hall Pharmacology

PROGRAMME

June 15th; 2012, Lecture Hall Pharmacology

2:00 – 4:30 p.m.

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Oral Presentation

Epithelial to Mesenchymal Transition (EMT) Leads to Docetaxel Resistance in Prostate Cancer and is Mediated by Reduced Expression of miR-200c and miR-205

Dr. Martin Puhr
Universitätsklinik für Urologie

1st funding period
MUI-START 2
Duration: 13 May 2011 – 12 May 2013

Chemotherapy with docetaxel is given to prostate cancer patients after endocrine therapy failure but in many cases its application is limited due to occurrence of an acquired resistance. Since EMT has been associated with progression of various cancers, we hypothesized that this process may be relevant for chemotherapy resistance. To uncover key mechanisms of docetaxel insensitivity we have established docetaxel resistant sublines.

In this study we report that docetaxel resistant cells showed a reduced expression of E-cadherin and an increased expression of mesenchymal markers, suggesting that prolonged docetaxel treatment causes EMT. Reduction of E-cadherin was mediated by decreased microRNA 200c and -205 expressions and could be reversed upon transfection of both microRNAs. EMT was in addition associated with an increased stem cell-like cell population and an aggressive cancer cell phenotype in vitro. Furthermore, analysis of tissue samples from patients who underwent neoadjuvant chemotherapy revealed a diminished E-cadherin expression, suggesting a similar situation in the patients. Moreover, reduced E-cadherin expression in patients after chemotherapy could be linked to tumor relapse.

The present study uncovers epithelial to mesenchymal transition as a hallmark of docetaxel resistance. Therefore, we suggest that this mechanism is at least in part responsible for chemotherapy failure with implications for the development of novel therapeutics.

Oral Presentation

Nuclear orphan receptor NR2F6 suppresses CD4$^+$ Th17 cell differentiation by antagonizing NFAT binding to the *Il17a* promoter

Dr. Natascha Hermann-Kleiter
Sektion für Zellgenetik

1st funding period
MUI-START 1
Duration: 22 April 2011 – 21 April 2012

Th17 cells have been tightly linked to autoimmune responses, nevertheless the detailed interplay of the transcriptional network regulating IL-17A cytokine expression is incompletely understood. We show that the nuclear orphan receptor NR2F6 interferes with the DNA binding capacities of NFAT and as well as other crucial transcription factors at the *Il17a* promoter as well as its CNS2 region. NR2F6 directly interacts with NFAT in a DNA scaffold dependent fashion dependent on both its DNA and ligand binding domain. In addition NR2F6 binds to the HRE elements within the distal and proximal *Il17a* promoter and thereby interferes with DNA accessibility of other crucial transcription factors. Consistent, binding of transcription factors within the *Il17a* promoter is enhanced in nuclear extracts derived from NR2F6-deficient CD4$^+$ Th17 cells but decreased in Th17 cells with forced NR2F6 over-expression. *In vivo* the negatively T cell intrinsic role of NR2F6 was confirmed by adaptive transfer EAE. These findings indicate an antagonistic role of the activating transcription factors versus NR2F6 during Th17 differentiation. We therefore propose that NR2F6 represents a promising molecular target for specific immuno-intervention in Th17-mediated autoimmune diseases.

Oral Presentation

A new role for Caspase-2 in embryonic development

Dr. Claudia Manzl
Sektion für Allgemeine Pathologie

1st funding period
MUI-START 2
Duration: 01 March 2011 – 28 February 2013

It is well known that female p53-defienct mice die in utero due to a neuronal tube closure defect. We could find out that additional lack of the enigmatic initiator-caspase caspase-2 leads to a partial rescue of the p53 null female pups. $p53^{-/-}$caspase-$2^{-/-}$ DKO mice were born with a frequency of 32% and included 20% female and 80% male double-deficient mice. In contrast, parallel p53-breedings ($p53^{-/-}$ x $p53^{+/-}$) yielded the expected 100% of male knockout pups of this genotype. Preliminary examination of embryos lacking p53 or caspase-2/p53 showed that, at a late embryonic stage of E17, 56 and 33% of the female knockouts developed exencephaly, respectively. These data indicate that female $p53^{-/-}$ mice die not only due to exencephaly but also due to other or additional developmental malformations. Interestingly, analyzing expression of genes involved in neuronal tube closure using cDNA derived from wt, $p53^{-/-}$ and DKO animals show that expression of the bone morphogenetic protein 2 (bmp2) is upregulated in brains from $p53^{-/-}$ animals compared to wt and DKO. Although it is reported that apoptosis is not required for correct process of neuronal tube closure, using western blot analysis of brain lysates from DKO mice we could monitor an activation of caspase-3, which is missing in lysates isolated from wt and $p53^{-/-}$ mice.

Altogether our data strongly support that caspase-2 has a function in embryonic development, e.g. in neural crest migration and in process of programmed cell death on a p53-deficient background.

Oral Presentation

Therapeutic angiogenesis by the neuropeptide catestatin

Dr. Markus Theurl
Innere Medizin I

1st funding period
MUI-START 2
Duration: 14 February 2011 – 13 February 2013

Introduction: We found that the neuropeptide catestatin (CST) influences endothelial cell (EC) functions and therefore hypothesized that it might induce angiogenesis.

Methods/Results: *In-vitro* matrigel experiments revealed that CST induces capillary tube formation comparable to classic angiogenic factors like basic fibroblast growth factor (bFGF). The observed effect could be blocked by a neutralizing bFGF-antibody (Ab) (CST 2.05±0.08, bFGF 2.25±0.09, CST+bFGF-Ab 1.1±0.05; n=4, P<0.01 CST vs. CST+bFGF-Ab). Interestingly, bFGF mRNA was not up-regulated by CST but we found that EC stimulated by CST release, MAPK dependent, bFGF into cell supernatant [pg/ml: Ctr 26.6±2.2, CST 53.8±2.4, PD±CST 21.2±1.04, P<0.01 CST vs. Ctr and CST vs. PD+CST,n=4].

Immunoprecipitation-studies revealed phoshorylation of FGF-receptor-1 after treatment of EC with CST. Western blot assays showed a biphasic activation of the ERK 1/2 signaling pathway by CST with a fast activation at 10 minutes and a later activation at 50 minutes. The late activation could be blocked by a bFGF-Ab indicating that bFGF might be important for a continuous activation of the MAPK-pathway in CST induced actions. To investigate an involvement of bFGF in CST induced angiogenesis *in-vivo* mice were subjected to hind-limb ischemia operation and treated with CST (20 µg every other day in adductor muscle for 4 weeks) and with either a neutralizing bFGF-Ab or IgG. FGF-Ab impaired limb reperfusion (LDPI ratio ischemic/non ischemic limb: CST+IgG 0.75±0.03 vs. CST+bFGF-Ab 0.46±0.04; n=6; P<0.01) and increased necrosis score (CST+IgG 1.14±0.14 vs. CST+bFGF-Ab 2.16±0.28; n=6; P<0.05) compared with IgG.

Conclusion: CST induced angiogenesis is mediated by a bFGF-dependent mechanism.

Oral Presentation

Assessment of normative values of motor activity during sleep: a video-polysomnographic study in a representative Tyrolean population sample

Priv.-Doz. Dr. Birgit Frauscher, Dr. David Gabelia
Universitätsklinik für Neurologie

1st funding period
MUI-START 2
Duration: 22 April 2011 – 21 April 2013

Background: Abnormal motor activity during sleep is a hallmark of many sleep disorders. Nevertheless, normal or abnormal has been defined only partially for some disorders, and not at all for others. Other motor phenomena during sleep have been described, but their prevalence in the normal population is completely unknown.

Aims: We aimed to establish normative video-polysomnographic values of motor phenomena during sleep in a sample representative of the Tyrolean population.

Methods: One-hundred healthy sleepers (59 women, 41 men) between 18–80 years of age underwent a comprehensive sleep interview and video-polysomnographic registration. Scoring of sleep macrostructure, arousal scoring and polysomnographic analysis of sleep-related motor activity were performed manually according to published methodology.

Results: Participants spent a median of 6.7 hours asleep during polysomnographic registration. Sleep efficiency was 86.4%. Median sleep latency was 14 minutes, REM latency 120 minutes. Median percentages of sleep stages were 11.8% N1, 52% N2, 20.5% N3, and 15.5% REM. Total arousal index was 13.4/h. Many previously reported motor phenomena during sleep were present: every patient had fragmentary myoclonus, 56% had neck myoclonus during REM sleep, 33% had high frequency leg movements, and 18% had periodic leg movements in sleep indices above 15/h. Median REM-related EMG activity indices were 12.8% for any EMG activity in the mentalis, and 19.7% for the mentalis plus the flexor digitorum superficialis muscles.

Conclusions: Our data provide both qualitative and quantitative normative values for different motor phenomena during sleep, and will therefore substantially contribute to a better differentiation towards pathological sleep-related motor phenomena.

Oral Presentation

A prospective study on metabolic risk factors and incidences of primary liver and gallbladder cancers in 578,700 adults in the Me-Can collaborative study

Dr. Wegene Borena
Sektion für Virologie

1st funding period
MUI-START 1
Duration: 01 March 2011 – 29 February 2012

Background: Few prospective studies have assessed the association between metabolic syndrome and hepatobiliary cancers.

Aim: to investigate the association between metabolic risk factor components, namely body mass index (BMI), blood pressure, glucose, cholesterol and triglycerides (individually and combined) and risks of primary liver and gallbladder cancers.

Methods: The metabolic syndrome and cancer project (Me-Can) includes cohorts from Norway, Austria, and Sweden with data on 578,700 subjects. We used Cox proportional hazard models to estimate relative risks (RRs) of primary liver and gallbladder cancer. RRs were corrected for random error in measurements.

Results: For primary liver cancer RRs per unit increment of z-score adjusted for age, smoking status and BMI and stratified by birth year, sex and sub-cohorts, were for BMI 1.39 (95% confidence interval (CI) 1.24–1.58), mid blood pressure 2.08 (0.95–4.73), blood glucose 2.13 (1.55–2.94), cholesterol 0.62 (0.51–0.76) and serum triglycerides 0.85 (0.65–1.10). The RRs per one unit increment of the metabolic syndrome (MetS) z-score was 1.35 (1.12–1.61). Similarly for primary gallbladder cancer, the respective RRs were for BMI 1.31 (1.11-1.57), mid blood pressure 0.96 (0.71-1.31), blood glucose 1.76, (1.10- 2.85), cholesterol 0.84 (0.66-1.06) and serum triglycerides 1.16 (0.82-1.64). The respective RR per one unit increment of the MetS z-score was 1.37 (1.07-1.73).

Conclusion: BMI, glucose and a composite MetS score were positively associated with risks of primary liver and gallbladder cancers. Cholesterol was negatively associated with primary liver cancer risk.

Oral Presentation

The biomechanical effects of a deepened articular cavity during dynamic motion of the wrist joint

Priv.-Doz. Rohit Arora, Dr. Stefanie Erhart
Universitätsklinik für Unfallchirurgie

1st funding period
MUI-START 1
Duration: 14 March 2011 – 13 March 2012

Background: A deepened articular cavity of the distal radius due to a metaphyseal comminution zone is associated with early osteoarthritis and reduced joint motion. As this deformity has not been investigated biomechanically, the purpose of this study was to evaluate the effects of a deepened articular cavity on contact biomechanics and motionrange in a dynamic biomechanical setting.

Methods: Six freshfrozen cadaver forearms were tested in a force controlled testbench during dynamic flexion and extension and intact mean contact pressure and contact area as well as range of motion were evaluated. Malunion was then simulated and intraarticular as well as motion data were obtained. Intact and malunion data were compared for the scaphoid and lunate facet and the total radial joint surface.

Findings: Due to malunion simulation, cavity depth increased significantly. Motion decreased significantly to 54-69% when compared to the intact state. Malunion simulation led to a significant decrease of contact area in maximum extension for all locations (by ~50%). In maximum flexion and neutral position, contact area decrease was significant for the scaphoid fossa (by 51-54%) and the total radial joint surface (by 47-50%). Contact pressure showed a significant increase in maximum extension in the scaphoid fossa (by 129%).

Interpretation: Already a small cavity increase led to significant alterations in contact biomechanics of the radiocarpal joint and to a significant range of motion decrease. This could be the biomechanical cause for degenerative changes after the investigated type of malunion. We think that restoration of the normal distal radius shape can minimize osteoarthritis risk post trauma and improve radiocarpal motion.

Oral Presentation

NMDA-receptor mediated excitotoxicity is involved in the pathogenesis of experimental cerebral malaria

Dr. Peter Lackner
Universitätsklinik für Neurologie

1st funding period
MUI-START 2
Duration: 01 April 2011 – 31 March 2013

A major cause of morbidity and mortality of Plasmodium falciparum malaria is cerebral malaria (CM). Mortality is high and neurological sequelae are frequently observed in survivors. In addition to ischemia and inflammation, excitotoxicity might be an important factor. The current study investigates the role of NMDA-receptor mediated cell death during CM.

C57BL/6J mice were infected intraperitoneally with $1x10^6$ Plasmodium berghei parasitized red blood cells. Cerebral microdialysis was performed and glutamate levels were measured. Animals with CM were randomized for treatment with artesunate, MK801 (a NMDA-receptor antagonist), MK801/artesunate or vehicle over 5 days. Clinical outcome was scored and brains were processed for Immunohistochemistry.

Glutamate levels were significantly elevated in mice with CM compared to control animals. Glutamate peaks were noted before and after clinical signs of CM developed. No animals survived in the MK801 or vehicle treatment group. In contrast, 33.3% the animals in the artesunate and 74.1% in the MK801/artesunate treatment group survived. Kaplan-Meier curves yielded a significantly longer survival in the combination-treatment group compared to the vehicle or MK801 treatment group. In addition MK801 treated animals showed significantly prolonged survival compared to vehicle treated animals, although cumulative survival was 0%. Histological analyses yielded a lower number of microhemorrhages and Fluoro-Jade B positive cells in the MK801/artesunate treated animals compared to artesunate treated mice.

In conclusion, glutamate levels in the brain are increased early in the course of CM. Treatment with an NMDA-receptor antagonist rescues mice from CM and could represent a target for adjunctive treatment strategies.

Poster Presentation

Role of Satb2 in postmitotic neuronal plasticity

Dr. Galina Apostolova
Gemeinsame Einrichtung für Neurowissenschaften

1st funding period
MUI-START 2
Duration: 01 March 2011 – 28 February 2013

Satb2 is a transcriptional regulator that binds to AT-rich DNA sequences of multiple gene loci, alters chromatin architecture and targets chromatin remodeling/modifying complexes. Based on our previous findings demonstrating a role of Satb2 in PNS neurotransmitter plasticity; and the expression pattern of Satb2 in adult CNS we hypothesize that Satb2 is implicated in the cellular mechanisms of synaptic plasticity as a regulator of activity-driven transcription.

In support of this hypothesis we first provide evidence that Satb2 level in CNS neurons is a correlate of increased neuronal activity both in vitro and in vivo. Treatment of primary hippocampal cultures with BDNF or increased synaptic activity (induced by Bic/4-AP application) causes an up-regulation of both Satb2 mRNA and protein as revealed by qRT-PCR and immunoblotting. Cued and contextual fear conditioning as an associative learning paradigm triggers alterations in Satb2 levels within the hippocampus - an increase at an early time point (1h) and a decrease at a late time point (24h). The increase at the early time-point coincides with previously reported hyper-phosphorylation of p42/44MAPK. Taken together, our results indicate that both endogenous excitatory synaptic activity and BDNF are sufficient to drive neuronal Satb2 expression. We are currently investigating the gene expression programs downstream of Satb2 in hippocampus/cortex by employing bioinformatics approaches, such as transcriptome profiling and deep sequencing.

To provide in vivo evidence supporting a role of Satb2 in CNS synaptic plasticity we will study neuronal gene expression, synaptic function, behavioral learning and memory in conditional Satb2 knockout models.

Poster Presentation

The heme oxygenase-1 system and long-term organ graft survival-'tolerance'

Ao. Univ.-Prof. Dr. Robert Öllinger, Dr. Kurt-Heinz Stromberger
Universitätsklinik für Visceral-, Transplantations- und Thoraxchirurgie

1st funding period
MUI-START 2
Duration: 01 April 2011 – 31 March 2013

Background & Aims: It is feasible to induce tolerance to allogenic organs by overexpression of heme oxgenase-1 (HO-1) or the administration of bilirubin in a murine heart transplant model. For both, donor specific blood transfusion (DST) is obligatory. Not much is known about the mechanism of DST. We investigate how DSTs together with HO-1 induction/bilirubin administration affect cytokine expression.

Methods: Resveratrol (as HO-1 inductor) and/or bilirubin are being administered i.p. together with a single i.v. DST (1^7 million cells; C57Bl/6) after murine heart transplantation (C57Bl/6-BALB/c). No further treatment is carried out. Graft function is evaluated by palpation. At defined timepoints (0, 1, 3 7 days) PCR is carried out for IL-6, IL-10, TNF and HO-1 in grafts, spleens and lymph nodes. Kaplan Meier and ANOVA are used for statistical analysis.

Results: Recipients receiving DST plus resveratrol showed significantly better graft survival (15d vs. 8d; p=0,03 vs. control). However, when DST was given allone, graft survival was similarly prolonged (14d). Cytokine expression was differently affected by DSTs and resveratrol. DST allone was the most potent inductor of HO-1 in the hearts, spleens and lymphatic nodes. TNF expression was maximally suppressed 72h after DST administration.

Conclusions: DSTs allone, that may become a clinical strategy facilitating tolerance induction in humans, does prolong survival and influence cytokine expression after heart transplantation in mice, possibly via HO-1 induction in the grafts. Whether an additive effect of HO-1 induction via resveratrol or bilirubin can be achieved and this occurs by differnt pathways has to be determined.

Poster Presentation

Targeted local therapy to overcome skin rejection in reconstructive transplantation

Dr. Theresa Hautz
Universitätsklinik für Visceral-, Transplantations- und Thoraxchirurgie

2nd funding period
MUI-START 2
Duration: 20 October 2011 – 19 October 2013

Background: Skin rejection is the major obstacle in reconstructive transplantation. Successful development of efficacious localized immunosuppressive therapy would enable to spare systemic immunosuppression and make reconstructive transplantation a suitable,safe option for patients suffering from severe tissue defects or inborn deformities.

Aim: To investigate the effect of local blockers (anti-IL-1b) on skin rejection in composite tissue allografts.

Methods: As adhesion molecules were significantly upregulated in human hand allografts during skin rejection, adhesion molecule blockers (E-+P-selectin blocker efomycine-M, anti-ICAM-1+anti-LFA-1) were given locally in combination with low-dose-immunosuppression in a rat hind-limb-transplant-model. A cell-isolation protocol of rat skin was successfully established for quantification of infiltrating cells using flow cytometry analysis. 230 rat allograft skin biopsies were assessed by multiplex-ELISA for expression of 14 cytokines associated with inflammatory/immune processes.

Results: After weaning tacrolimus on POD 50 animals rejected on POD 61±1 (grade III). Additional treatment with local efomycine-M or anti-LFA-1+anti-ICAM-1 resulted in long-term (150 days) allograft survival in 4/5 and 3/4 animals, respectively. Characterization of rejection in rat allograft skin revealed about 60% CD45+CD3+CD4+T-cells and 40% CD45+CD3+CD8+T-cells, dependent on the site of biopsy sampling. A defined set of inflammatory cytokines preferentially expressed in allografts vs isografts vs controls were identified. IL-1b was recognized to be significantly upregulated in allografts, but not isografts.

Conclusion: Local administration of adhesion molecule blockers result in significant prolongation of graft survival after total withdrawal of systemic immunosuppression. As IL-1b was identified as a key molecule during skin rejection, an IL-1b blocker is currently investigated for its local effect in a rat CTA model.

Poster Presentation

Preconditioning with tetrahydrobiopterin saves aortic allografts from chronic vasculopathy

Dr. Rupert Oberhuber
Universitätsklinik für Anästhesie und Intensivmedizin

2nd funding period
MUI-START 2
Duration: 22 July 2011 – 21 July 2013

Chronic allograft vasculopathy (CAV) has deleterious impact on long-term graft survival. Early oxidative stress caused by ischemia reperfusion injury (IRI) is one oft the most important risk factor for the development of CAV. Herein we examined whether tetrahydrobiopterin (BH4) an essential cofactor of nitric oxide synthases and strong antioxidant attenuates CAV preventing IRI.

A fully MHC mismatched (BALB/c to C57BL/6) murine cervical aortic transplantation model was used. Transplanted grafts were subjected to 24h cold ischemia time (CIT). Donor animals received either BH4 (50mg/kg b.w.) or saline. Aortas without CIT as well as syngeneic animals served as controls. IRI associated oxidative stress and parenchymal damage was assessed by means of glutathione tissue levels and CD-31 immunohistochemistry ten hours following reperfusion. Concentric intimal hyperplasia and endothelial cell activation was quantified by histopathology and immunohistochemistry (alpha-smooth muscle actin, E-selectin, P-selectin and ICAM-1) as early as 4 weeks following reperfusion. BH4 tissue levels within the aortic grafts were measured by HPLC.

After donor treatment with BH4 detected tissue levels were substantially increased, when compared to the untreated animals. Prolonged CIT resulted in a significant reduction of glutathione tissue levels in the untreated group ($p<0.05$) whereas BH4-treatment significantly restored glutathione tissue levels ($p<0.05$). Furthermore, reduced CD-31 expression following CIT was abrogated by BH4. Four weeks following transplantation prominent concentric intimal hyperplasia was observed in the untreated group but not following pre-treatment with BH4 ($p<0.001$), which, by contrast, was comparable to syngeneic controls and grafts without CIT. These findings were confirmed by immunohistochemistry. Treatment with BH4 elicits a significant reduction of α-SMA positive cells within the intima. Finally, reduced expression of E-selectin, P-selectin and ICAM-1 was detected in aortic grafts treated with H4B.

Our experimental data point towards the strong correlation between IRI and CAV development. A single time application of BH4 might therefore represent a promising novel strategy to prevent CAV.

Poster Presentation

Effects of Vasopressin on migration and oxygen free radical release of human leukocytes

Dr. Martina Stichlberger
Universitätsklinik für Anästhesie und Intensivmedizin

2nd funding period
MUI-START 2
Duration: 15 September 2011 – 14 September 2013

Keywords: Vasopressin, leukocyte migration, chemotaxis, respiratory burst, sepsis

Background: Vasopressin, a hypothalamic nonapeptide, is wellknown for its effects on vasoconstriction, platelet aggregation and water resorption in renal collecting ducts. It is also involved in immune modulation jointly with other catecholamines. Vasopressin stimulates together with corticotropin releasing hormone the production of adrenocorticotropic hormone (ACTH) and thus corticosterioid production. Moreover Vasopressin has also direct pro- and anti-inflammatory effects on human leukocytes. In vitro Vasopressin induces IFN-γ production and enhances lymphocyte response. It also acts as a chemoattractant for small cell lung carcinoma cells and possibly for monocytes similar to bombesin. On the other hand vasopressin did not stimulate the migration of polymorphonuclear leukocytes and also failed to enhance superoxide anion release of macrophages in comparison to substance P. In vasopressin-deficient rats with permanently reduced number of blood leukocytes and decreased macrophage activity the administration of vasopressin resulted in enhanced phagocytosis of peritoneal macrophages. Increased Vasopressin production may indicating chronic inflammatory disease. On the other hand vasopressin may limit immunresponse as illustrated in other studies (eg in urinary tract infection vasopressin down-regulates chemokine secretion)

Objective: To elucidate potential benefits of vasopressin administration in patients with shock, we want to investigate the effects of vasopressin on migration, chemotaxis of human peripheral mononuclear cells, monocytes and neutrophils and the influence of vasopressin on oxygen free radical release of human neutrophils in vitro.

Methodology: Laboratory study

Co-operating partner: This study is performed in cooperation between the Department of Anaesthesiology and Critical Care Medicine and the Department of Internal Medicine Innsbruck.

Time schedule: The project started some weeks ago with detecting the neutrophil respiratory activity. In August we will begin our chemotaxis experiments and depending on the results of our preliminary investigation further experimental and clinical research are envisaged to assess vasopressin's influence on leukocytes function in patients with SIRS and septic shock.

Poster Presentation

Cell death mechanisms in adipogenesis

Dr. Christian Ploner
Plastische, Rekonstruktive und Ästhetische Chirurgie

1st funding period
MUI-START 2
Duration: 01 January 2011 – 30 June 2012

Background: One of the most interesting features of adipocytes remains their longevity that exceeds almost 10 years. After passing their lifespan, adipocytes undergo cell death resulting in numerous cell fragments and lipid droplets absorbed by infiltrating macrophages. Thus, increased adipocyte cell death can cause chronic inflammation which becomes clinically relevant in obesity-associated disorders like type II diabetes. Although research on adipose tissue physiology has been intensified over the last years, knowledge on cell death regulation in adipose tissue is still limited.

Aims: To determine the functional significance of pro-survival BCL2 proteins for ASC survival during differentiation and define their possible role in autophagy dependent depletion of mitochondria during adipogenesis.

Methods: All experiments have been performed using primary human ASC isolated from fat specimen of patients undergoing plastic surgical intervention. We analysed expression of prosurvival BCL2 proteins in primary ASC and differentiated adipocytes on mRNA (quantitative RT-PCR) and protein level (immunoblotting). Further we applied lentivirally transduced conditional RNAi to knock down MCL1, BCL2 and BCLXL and subjected KD and control cells to in-vitro differentiation and survival assays.

Results: We observed significant induction of BCL2 and BCLW in in-vivo differentiated adipocytes. In contrast, MCL1 and BCLXL levels were decreased in these cells. Interestingly, MCL1 and BCLXL levels remained constant during in-vitro differentiation, whereas BCL2 and BCLW levels were induced similarly to in-vivo differentiated adipocytes. To determine the functional significance of these regulations for ASC survival and differentiation, we performed KD experiments of MCL1, BCLXL and BCL2. These data revealed that the KD of BCLXL, but not MCL1 or BCL2 induced enhanced cell death in ASC. Same cells subjected to in-vitro differentiation assays showed decreased differentiation of BCLXL KD, but increased differentiation of MCL1 KD and a moderate decrease in BCL2 KD cells.

Conclusions: Although we investigated only a limited number of patients (n=4), we propose following model: high BCL2 levels in adipocytes are essential to protect the adipocyte mitochondria, MCL1 (co-) regulates early adipocyte differentiation and BCLXL expression is essential for ASC survival.

Poster Presentation

A non-coding RNA expression library for the functional analysis of proliferation and differentiation of human adipocyte derived stem cells

Dr. Andreas Ploner
Sektion für Genomik und RNomik

2nd funding period
MUI-START 2
Duration: 01 August 2011 – 31 July 2013

In recent years, several hundreds of new small non coding RNAs (ncRNAs) from various species and organisms were identified through either experimental or computational approaches. NcRNAs are not translated into proteins but act as *genetic switches* in the regulation of fundamental cellular processes, including transcriptional regulation, RNA processing and modification, messenger RNA stability or protein translation. Thus, the functional investigation of these genetic switches is an important step towards our understanding of the complex cellular networks.

White adipose tissue (WAT) is the primary site of energy storage within the human body and composes about 20 to 25 % of the body weight. Beside triglyceride synthesis from lipoprotein-derived fatty acids, hormone-stimulated glucose uptake and lipolysis, WAT is a highly endocrine organ, required for the expression and secretion of adipokines, playing a key role in the pathogenesis of obesity, diabetes and other metabolic diseases. Recent studies suggest that approximately 10% of the body's fat cells are regenerated each year, thus adipogenesis has to be tightly controlled on both, transcriptional as well as on translational level.

In this study we want to identify new regulators of differentiation and proliferation in adipogenesis by generating a expression library form small non coding RNAs. We will provide new insights in the regulation process of human adipocyte differentiation thus giving yet unknown knowledge in the development of obesity and furthermore arise new targets for its treatment.

Poster Presentation

Drug target analysis in the pulmonary orphan disease pulmonary arterial hypertension

Dr. Katharina Cima
Innere Medizin I

1st funding period
MUI-START 2
Duration: 15 April 2011 – 14 April 2013

Pulmonary arterial hypertension (PAH) is still incurable and thus a life threatening disease. The sustaining elevated pressures in the lung are currently believed to be caused by mechanisms such as vasoconstriction, vascular remodelling and thrombotic formations that occur due to impaired regulation of pulmonary artery endothelial cells (PAEC), pulmonary artery smooth muscle cells (PASMC) and endothelial progenitor cells (EPC). However, the to-date available medication can only ameliorate the symptoms and thus, there is an urgent need to develop new therapeutic strategies against PAH. As the pathophysiological mechanisms of PAH quite resemble those occurring in cancer, one new approach focuses on antiproliferative and proapoptotic agents that target receptor kinases.

Initially developed for non small cell lung cancer (NSCLC) treatment, our aim is to analyse the efficacy of receptor kinase inhibitors such as sorafenib and regorafenib in the key cells of the orphan lung disease PAH.

Male Sprague Dawley rats of 6-8 weeks old were treated with monocrotaline (MCT) for 3-4 weeks. EPC were isolated from the bone marrow and by microsurgery the PAEC and PASMC from large and small pulmonary arteries of healthy and diseased rats were isolated at the ECCPS of Gießen, Germany. After establishing the cell cultures, isolated cells were treated with tyrosine kinase receptor inhibitors, sorafenib [10^{-5} M to 10^{-6} M] and regorafenib [10^{-6} M to 10^{-8} M] for 24 or 48h, respectively. So far, cell proliferation assays were carried out by applying WST-1. For assessing cell viability trypan blue viability assays were performed.
For this project, the techniques of the most common PAH animal model have been acquired at the ECCPS in Gießen, Germany. Furthermore, for the first time, EPC of MCT rats could be isolated, the protocol for PAEC isolation could be enhanced and finally, cell cultures of EPC, PAEC and PASMC could be established.

Sorafenib [10^{-5} M] and regorafenib [10^{-6} M] showed to inhibit cell growth of EPC and SMC most significantly (***, $p<0.0001$). Interestingly, the effects of sorafenib and regorafenib did not vary between small and large pulmonary artery SMC. The trypan blue assay also revealed both substances to affect cell viability. Regorafenib treatment of EPC revealed a viability of 22.5 to 37%; by contrast, sorafenib displayed an EPC viability of 15 to 48%. Furthermore, PASMC of small and large vessels displayed a growth arrest upon treatment, the viability ranging between 25-50%. (All viability rates depending on the agents' concentration).

In sum, a further step has been taken in exploring sorafenib and regorafenib and their effects in PAH, showing promising results. As next steps FACS analyses are planned to determine cell growth arrest apart from analyzing cell functions such as cell migration and angiogenesis and the involved downstream signaling pathways.

Poster Presentation

PhoG - a transcription factor regulating adaptation to limitation and stresses in *A. fumigatus*

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1st funding period
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Background: Aspergillus fumigatus is the most prevalent airborne fungal pathogen and can cause severe diseases or even death in intensive care patient, featuring mostly a debilitated immune system. The infection process implies an adaptation to the host environment like carbon, nitrogen and nutrient starvation. In Aspergillus nidulans, the transcriptional activator PhoG (XprG), which belongs to the Ndt80 family, is involved in the response to nutrient limitation; in Candida albicans deficiency in the corresponding homolog causes attenuation in virulence.

Aim: Aspergillus fumigatus ATCC46645 possesses two PhoG paralogs, PhoG1 and PhoG2. Aim of the project is the functional analysis of the role of these transcription factors in A. fumigatus ATCC46645 in adaptation to nutrient limitation, stress response and virulence.

Methods: The function of the two PhoG paralogs is analysed by single and double gene inactivation followed by phenotypic analysis of the mutant strains under various nutrient-limiting conditions.

Results: The generation of the single and double mutant strains was successful. Inactivation of PhoG1 decreased peptone-induced proteolytic extracellular activity, oxidative stress resistance and conidiation. In contrast, inactivation of PhoG2 slightly increased extracellular proteolytic activity. In activation of PhoG2 and particularly simultaneous inactivation of PhoG1 and PhoG2 was deleterious in adaptation to iron limitation.

Conclusion: PhoG-type transcription factors are involved in adaption to nutrient limitation in A. fumigatus. As adaptation to nutrient limitation is crucial for virulence, in a next step the putative role of these regulators in virulence will be studied.

Poster Presentation

Molecular characterization of ribosomal E-site function

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Ribosomal protein biosynthesis is a complex multistep process of translation of the genetic code encoded on the mRNA into proteins. This process is universally conserved and the main target for the majority of natural antibiotics. During the elongation cycle of translation tRNAs travel through the ribosome by consecutive binding to three ribosomal tRNA binding sites, the A-, P- and E-site. While the ribosomal A- and P-sites have been functionally well characterized in the past, the contribution of the E-site is still poorly understood in molecular terms. Two 2'-OH groups at positions 71 and 76 in the acceptor stem of the tRNA have been found to be crucial for tRNA translocation from the P- to the E- site. Equipped with the recent high resolution ribosome structures and our atomic mutagenesis approach, potential interaction partners in the E-site of the two important OH groups can now be experimentally characterized.

Atomic mutagenesis has already successfully been used to gain deeper insights into peptide bond formation and peptidyl tRNA hydrolysis, translocation and EF-G GTPase activation on the ribosome. This tool is now used to identify the critical interaction partners of the two pivotal tRNA groups in the 23S rRNA on the molecular level. The key feature of this tool is the site-specific introduction of non-natural nucleoside analogs at any desired position within 23S rRNA. The modified rRNA is then reconstituted into ribosomes and the particles are tested in individual reactions of protein biosynthesis and also employed in the overall performance during in vitro translation. Data obtained from these studies will allow us to functionally characterize the role of distinct chemical groups on the 23S rRNA involved in tRNA translocation and maintaining the E-site tRNA in a productive conformation.