Biomedical Symposium

for Graduate Students

November 9-10th, 2012

BIOMEDIGS

BIOMEDICAL INTERNATIONAL GRADUATE SCHOOL

Regensburg
International Graduate School
of Life Sciences

Biomedical Symposium for Graduate Students
November 9th and 10th, 2012
Tagungshaus Bayerischer Wald, Regen

Organisation: Johanna Canady
Benedikt Groschl
Anke Rudel

Cover photo: Steven Keller

PROGRAM BIOMEDIGS Symposium 2012

FRIDAY, 9th of November 2012

8.00-9.30	**DEPARTURE from the Klinikum Bus Stop**
9.30-10.00	**CHECK-IN**
10.00-10.30	**RECEPTION**
10.30-12.00	**TALKS PART I**

IFN-γ-induced iNOS Expression in Mouse Regulatory Macrophages Prolongs Allograft Survival in Fully Immunocompetent Recipients
Anja Kammler

The functional role of AP-2ε in chondrocytes and osteoarthritis
Stephan Niebler

Regulationn of the increased N-Cadherin levels in Osteoarthritis synovial fibroblasts
Anke Rüdel

Ultrastructural and cell-biological studies of the primary cilium
Benjamin Salecker

MicroRNAs in the kidney and their relevance for podocyte (dys)function
Susanne Baumgarten

12.00-13.00	**LUNCH**

13.00-14.00 **POSTER** **PART I**

Genetic suppression of lactate dehydrogenase A (*Ldha*) in murine B16.SIY melanoma cell line modulates immune cell infiltration and reduces tumor growth *in vivo*
Almut Brand

DUSP4 Expression Is Associated with Microsatellite Instability in Colorectal Cancer (CRC) and Causes Enhanced Cell Growth
Benedikt Gröschl

A mutation in a mitochondrial protein causes renal Fanconi syndrome
Carsten Broeker

The role of the miRNA processing proteins Exportin 5 and Drosha in malignant melanoma
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Molecular mechanisms of early cancer dissemination in Balb-NeuT mice
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Enhanced renin cell recruitment in mice deficient for the cGMP-dependent protein kinase II
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The mechanisms of compensatory renal hypertrophy
- the role of B-type natriuretic peptide and the role of podocytes in GC-A-receptor-signaling pathway
Janina Staffel

The role of MTA in malignant melanoma
Katharina Limm

The influence of type XVI Collagen on cell growth and invasion in oral squamous cell carcinoma
Konstanze Bedal

TMEM16 proteins partially produce Volume-activated Chloride Current
Lalida Sirianant

14.00-14.30 **Industrial Tutorial QIAGEN**

14.30-15.00 **COFFEE BREAK with Group Picture**

15.00-16.00 **POSTER PART II**

Role of MIA signaling in malignant melanoma
Lena Honold

I**n the experimental model of leishmaniasis myeloid-derived suppressor cell functions and interaction with parasites differs between C57BL/6 and BALB/c**
Maximilian Schmid

Role of CYLD in early melanoma development
Miriam deJel

Molecular function of the liver protein MIA2
Mona Solanki

Characterization of bactericidal/permeability increasing protein expression and function based on newly developed transgenic mouse models.
Nicole Bezold

Anoctamin1 expression in carcinoma cells
Podchanart Wanitchakool

The role of histone modifications in the pathogenesis of glioblastoma and their relevance as a potential novel treatment option.
Sabine Hoja

DLX3 Regulates Apoptosis and the Osteogenic Differentiation of Human Dental Follicle Precursor Cells
Sandra D. Viale Bouroncle

ALR – a novel and auspicious target for NASH treatment?
Susannah Spieker

THE SPATIAL ENERGY EXPENDITURE CONFIGURATION AND POSSIBLE APPLICATIONS IN AN EXPERIMENTAL MODEL OF ARTHRITIS
Susanne Klatt

16.00-17.00 **MEET THE EXPERT**
Cristiano Ferlini, MD, PhD
Director Medical Research,
Danbury Hospital Research Institute

17.30-19.00 **DINNER**

19.30-open end **Wellness und GET TOGETHER**

SATURDAY, 10th of November 2012

9.00-9.30 **BIOMEDIGS Meeting**

9.30-10.30 **TALKS** **Part II**

Characterization of the anti-inflammatory impact of molecular fragments derived from the adipokine CTRP-3 in adipocytes and monocytes
Andreas Schmid

Creation and Characterization of *Pkd2* Knock-in Mice
Denise Schmied

The impact of the basolateral potassium channel KCNJ10 for salt resorption in the distal tubule of the kidney
Maria Ripper

The physiological and pathophysiological relevance of the inwardly rectifying potassium channel Kir5.1 (KCNJ16) in the kidney
Evelyn Humberg

10.30-11.00 **Coffee with Poster Walk/Evaluation**

11.00-12.00 **TALKS** **PART III**

Superficial Nephrons in BALB/c and C57BL/6 Mice Facilitate in vivo Multiphoton Microscopy of the Kidney
Ina Schießl

Increased KGF Expression Promotes Fibroblast Activation in a Double Paracrine Manner Resulting in Cutaneous Fibrosis.
Johanna Canady

The role of alpha Syntrophin in hepatic lipid metabolism
Kristina Eisinger

Molecular Function and Transport of MIA
Matthias Molnar

12.00-13.00 **LUNCH**

13.00-13.30 **INDUSTRIAL TUTORIAL**
"Life Technologies: Talent Acquisition"
Peggy Klein

13.30-14.30 **TALKS** **Part IV**

InSilico analysis of medical and biological data using the *SISSi* software
Torsten Schön

Utilizing Patient Specific Induced Pluripotent Stem Cell Derivatives to Functionally Characterize HSPB7 Cardiovascular Risk Alleles
Maya Fürstenau-Sharp

Connections between LMX1B-regulated proteins and the actin cytoskeleton
Natalya Lukajczyk

The impact of mutated Fanconi-associated protein on the mitochondrial proteome
Nadine Aßmann

Deletion of TASK3 K^+ channels leads to hyperaldosteronism in adrenal glands of neonatal mice
Philipp Tauber

14.30-15.00 **COFFEE BREAK**

15.00-15.30 **ELECTIONS BEST POSTER / BEST TALK**

15.30-16.00 **AWARD PRESENTATION and FAREWELL**

Talks Part I

IFN-γ-induced iNOS Expression in Mouse Regulatory Macrophages Prolongs Allograft Survival in Fully Immunocompetent Recipients

Anja Kammler Talk 1

The functional role of AP-2ε in chondrocytes and osteoarthritis

Stephan Niebler Talk 2

Regulationn of the increased N-Cadherin levels in Osteoarthritis synovial fibroblasts

Anke Rüdel Talk 3

Ultrastructural and cell-biological studies of the primary cilium

Benjamin Salecker Talk 4

MicroRNAs in the kidney and their relevance for podocyte (dys)function

Susanne Baumgarten Talk 5

IFN-γ-induced iNOS Expression in Mouse Regulatory Macrophages Prolongs Allograft Survival in Fully Immunocompetent Recipients

Anja Kammler, Paloma Riquelme, James A. Hutchinson, Edward K. Geissler

Background *The ONE Study* is currently investigating human regulatory macrophages (M reg) as a potential cell-based adjunct immunosuppressive treatment in renal transplantation. Using culture conditions analogous to those under which human Mregs arise, a mouse counterpart of human M regs has now been generated.

Results Mouse monocytes acquire an M reg phenotype over seven days in culture, during which time the cells are exposed to M-CSF and IFN-γ. Mouse M regs adopt a characteristic morphology and express markers distinguishing them from monocytes, monocyte-derived DCs and M0-, M2a-, and M2c-polarised macrophages. In suppression assays, M regs completely inhibit polyclonally-stimulated T cell proliferation through an inducible nitric oxide synthase (iNOS)-dependent mechanism. In a heterotopic heart transplant model, a single intravenous administration of $5x10^6$ donor-strain M regs prior to transplantation significantly prolongs allograft survival in unconditioned, non-immunosuppressed recipients using both the stringent C3H-to-BALB/c (32.6±4.5 vs. 8.7±0.2 days; $p<0.001$) and B6-to-BALB/c (31.1±12 vs 9.7±0.4 days; $p=0.002$) strain combinations. This graft-protective effect was specific to donor cells as recipient did not prolong graft survival compared to untreated controls (9.6±0.4 days; ns) and third party-derived M regs only very marginally prolonged graft survival (11.0±0.6 days; $p=0.004$). Co-treatment with M regs and 1 mg/kg/day rapamycin for 10 days post-transplant enhanced the effect of M regs (64.1±8.6 days) compared to M reg treatment alone ($p=0.006$) or rapamycin alone ($p=0.02$) and some recipients accepted grafts indefinitely. One day after administration, M regs were readily detected in the blood, spleen, liver and lung of both congeneic and allogeneic recipients, but were not reliably detected in lymph nodes or bone marrow. By the fourth week, no M regs could be reliably detected.

Conclusion Mouse M regs represent a novel, phenotypically distinct subset of macrophages which bear a close resemblance to human M regs in terms of their mode of derivation, marker phenotype and *in vitro* functions. Identifying and accurately characterizing mouse M regs has provided a convenient model to study the mechanisms of M reg action *in vivo* and also to define an optimal immunosuppressive context for the delivery of M reg therapy.

The functional role of AP-2ε in chondrocytes and osteoarthritis

Stephan Niebler, Anja K. Bosserhoff

AP-2 (activating enhancer-binding protein-2) transcription factors play fundamental roles in numerous physiological processes including development and differentiation as well as tumorigenesis. The AP-2 protein family consists of five members: AP-2α, AP-2β, AP-2γ, AP-2δ and AP-2ε. These isoforms are highly conserved nevertheless have non-redundant functions highlighted by AP-2 knockout (k.o.) mice. For regulation of target gene expression AP-2 proteins form homo- and heterodimers that bind to the palindromic DNA recognition sequence 5´-GCCN$_3$GGC-3´ within multiple promoters. Expression of AP-2ε, the last identified AP-2 isoform, was detected in brain and skin tissue. In previous studies we could reveal that AP-2ε is also expressed in chondrocytes and up-regulated in osteoarthritis (OA).

To better understand the function of AP-2ε in cartilage we screened for AP-2ε target genes in chondrocytes. Performing promoter analyses including reporter gene assays, chromatin immunoprecipitation, and electrophoretic mobility shift assays as well as quantitative real-time PCR we could confirm the genes for the cartilage specific type II collagen and the chemokine CXCL1 to be specifically regulated by the transcription factor. Both are associated with chondrocyte differentiation / hypertrophy and OA progression, respectively, suggesting a modulation of these processes by AP-2ε.

In contrast to AP-2 α, ß and γ k.o. mice, AP-2ε deficient mice show no obvious phenotype indicating compensatory mechanisms in tissues where AP-2ε is normally expressed. As it is known that in some cases abnormalities of k.o. specimen only become apparent under pathological conditions we established an animal model of OA by destabilization of the medial meniscus. Strikingly, OA development was significantly enhanced in AP-2ε k.o. mice compared to wt mice 17 days after OA onset. We found the matrix metalloproteinase MMP13, mediating collagen degradation, to be significantly up-regulated in AP-2ε deficient mice. This enzyme is known to promote OA development offering a plausible explanation for the enhanced disease progression observed in the k.o mice.

Taken together, our data reveal for the first time functions of AP-2ε in chondrogenesis, cartilage homeostasis and osteoarthritis development.

REGULATION OF THE INCREASED N-CADHERIN LEVELS IN OSTEOARTHRITIS SYNOVIAL FIBROBLASTS

Anke Ruedel, Klaus Stark, Anja K. Bosserhoff

Osteoarthritis (OA) is the most common form of arthritis and is characterized by the destruction of cartilage. OA fibroblasts of the synovial membrane are activated and are then, to some extent, able to migrate into the cartilage and destroy it. To identify differences between OASF and healthy synovial fibroblasts (SF) the mRNA expression pattern of known migration affecting genes, e.g. adhesion molecules were analyzed and we found that N-cadherin expression was highly increased in SF of OA patients. In general, N-cadherin is known to promote migration of tumor cells. But in some cancers, e.g. osteosarcomas, N-Cadherin has also been described as a tumor suppressor, by inhibiting migration. For further investigation of the role of N-Cadherin in OA, the *N-cadherin* promoter was screened for differences in OASF compared to SF from healthy donors. We were able to identify a SNP in the *N-Cadherin* promoter, which is associated with a positive outcome for OA patients. This SNP leads to the formation of new transcription factor binding sites and to an even higher N-cadherin expression. Calculations using the SNPInspectortool (Genomatix Software GmbH) revealed possible transcription factors which could bind to the altered binding site, as ETS-1. As several assays showed no differential binding of ETS-1 to the two SNP variants and ETS-1 was ruled out to be the responsible factor, a pull-down assay, using oligonucleotides for both variants was performed. After separation of the proteins on an acrylamide gel, differential bands were analyzed by mass spectrometry and hnRNP K was identified as a potential regulator of the increased N-Cadherin expression. Several assays, as EMSA and ELISA, showed that hnRNP K was only able to bind to the minor variant of the SNP and could therefore be the factor responsible for the increased N-Cadherin expression in patients carrying the minor allele of the SNP. In further studies we aim to clarify why OASF show a high expression of N-Cadherin and destroy cartilage while OASF carrying the minor variant of the SNP and show an even higher N-Cadherin expression are somehow protective in OA. In summary, these results show that the influence of N-cadherin on the aggressiveness of OASF is complex and requires further investigation.

Ultrastructural and cell-biological studies of the primary cilium

Benjamin Salecker, Karin Schadendorf, Cornelia Niemann, Larissa Osten, Christine Meese, Uwe de Vries, Reinhard Rachel, Ralph Witzgall

The non-motile primary cilium can be found on most mammalian cells. It is thought to act as a sensory organelle and plays a role as a signaling center during development. It is composed of a basal body and the ciliary shaft that extends into the extracellular space. Between the basal body and the shaft is the transition zone that is believed to form a barrier between the ciliary membrane and the cytoplasmic membrane. The basal body consists of 9 triplets of microtubules and distinct appendages around them, the basal feet, with the basal cap at their end. The axoneme of the ciliary shaft is made up of 9 pairs of microtubules. Mutations in genes encoding for ciliary proteins can cause a variety of diseases, the so called "ciliopathies". There are, amongst others, the Bardet-Biedl syndrome (BBS) and autosomal dominant polycystic kidney disease (ADPKD). ADPKD is caused by mutations in the *PKD1* or the *PKD2* genes. The *PKD2* product Polycystin-2 forms a cation channel and acts as a signal transduction molecule. BBS is caused by mutations in genes that encode the BBS proteins that are involved in ciliary transport.

We will further investigate the ultrastructure of the primary cilium especially by means of electron tomography. The use of high-pressure freezing and freeze substitution during sample preparation results in a better structural preservation with less artifacts. 3D-models of tomograms will be generated. Moreover the primary cilium of inducible knock-down mutants of ciliary and BBS proteins will be examined by electron tomography and the results will be compared to those of wild type cilia to find out which structural abnormalities are caused by the knock-down of the specific protein.

Of special interest are previous findings of Sonja Gürster who showed that vesicles can be found inside the basal body. To decide whether these vesicles represent anterograde or retrograde transport, different endocytosis experiments with electron microscopy as well as with fluorescence microscopy are to be done. One of these experiments led to the assumption that endocytosis might play a role in ciliogenesis. This will be investigated with additional experiments, again with fluorescence microscopy as well as electron microscopy. Therefore different proteins that are specific for different ways of endocytosis and intracellular transport will be checked for colocization with ciliary structures.

By now, the subcellular distribution of ciliary proteins is still uncertain and it is unclear to which structures they contribute. To answer these questions, correlative light and electron microscopy with the so-called miniSOG protein is used for protein localization of polycystin-2 and several BBS proteins that form the so called BBSome.

MicroRNAs in the kidney and their relevance for podocyte (dys)function

Susanne Baumgarten, G. Knoll, M. Herrmann, M. Billmeier, M. Zaparty, R. Witzgall

Introduction MicroRNAs are short, regulatory non-coding RNAs (19-25 nt) found in all eucaryotic cells. They are regulating several biological processes by guiding the so-called RNA-induced silencing complex (RISC) to their specific target mRNAs, thus leading to translational inhibition. Some miRNAs are highly enriched in certain cell types or at distinct developmental stages. Dysregulation of miRNA expression can contribute to the development of diseases.

Goal The aim of this work is to identify miRNAs important for the structure and function of podocytes. Beside functional analyses of specific miRNAs, we are interested in regulation of miRNA expression by the podocyte-specific transcription factor Lmx1b which is mutated in patients with nail-patella-syndrome (NPS), a genetic disorder that affects kidney filtration.

Methods and Results A validated set of glomerular miRNAs has been identified by deep sequencing, Northern blot and qRT-PCR analyses (collaboration with Prof. G. Meister). By using podocytes isolated from double-fluorescent Cre-reporter mice several of these glomerular miRNAs could be validated as truly podocyte-expressed.

In silico analyses were performed to predict target mRNAs coding for important structural podocyte proteins. Validation of these predicted miRNA-mRNA pairs is currently performed by Argonaute-immunoprecipitation (AgoIP) from a human podocyte cell line to precipitate the Ago-bound miRNAs and mRNAs. For functional analyses of miRNAs, a stable knockdown technique, “miRNA-sponges”, has been established in cell culture and, in addition, miRNA-overexpression studies will be performed. To investigate a putative role of miRNAs in NPS, we generated miRNA profiles of glomeruli derived from *Lmx1b* knockout mice by deep sequencing analyses (collaboration with A. Dück, Prof. G. Meister) which revealed a set of significantly down-regulated miRNAs. For verification that these miRNAs are regulated by Lmx1b, ChIP and overexpression analyses are currently performed, and EMSA studies are planned.

Perspective Global analyses using microarrays of the AgoIP mRNA will be performed to gather information about miRNA-regulated target genes in podocytes. To identify miRNA-targeted processes in podocytes, stable podocyte-specific *in vivo* expression of miRNA-sponges using lentiviral vectors is currently being established.

Poster Part I

Genetic suppression of lactate dehydrogenase A (*Ldha*) in murine B16.SIY melanoma cell line modulates immune cell infiltration and reduces tumor growth *in vivo*

Almut Brand Poster 1

DUSP4 Expression Is Associated with Microsatellite Instability in Colorectal Cancer (CRC) and Causes Enhanced Cell Growth

Benedikt Gröschl Poster 2

A mutation in a mitochondrial protein causes renal Fanconi syndrome

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The role of the miRNA processing proteins Exportin 5 and Drosha in malignant melanoma

Corinna Ott Poster 4

Molecular mechanisms of early cancer dissemination in Balb-NeuT mice

Hedayatollah Hosseini Poster 5

Enhanced renin cell recruitment in mice deficient for the cGMP-dependent protein kinase II

Ilona Schwarzensteiger Poster 6

The mechanisms of compensatory renal hypertrophy - the role of B-type natriuretic peptide and the role of podocytes in GC-A-receptor-signaling pathway

Janina Staffel Poster 7

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The influence of type XVI Collagen on cell growth and invasion in oral squamous cell carcinoma

Konstanze Bedal Poster 9

TMEM16 proteins partially produce Volume-activated Chloride Current

Lalida Sirianant Poster 10

Genetic suppression of lactate dehydrogenase A (*Ldha*) in murine B16.SIY melanoma cell line modulates immune cell infiltration and reduces tumor growth *in vivo*

Almut Brand, Katrin Singer, Gudrun Koehl, Michael Kastenberger, Gabriele Schoenhammer, Monika Wehrstein, Anna Hoehn, Eva Gottfried, Wolfgang Mueller-Klieser, Reinhard Andreesen, Marina Kreutz

The function of tumor-infiltrating immune cells is depressed and several tumor-derived factors have been implicated as potential mediators including cytokines and tumor metabolites like lactate. Lactate accumulates in the tumor microenvironment due to the accelerated glycolysis of tumor cells, a phenomenon known as “Warburg effect”. We and others have shown that lactate inhibits both the differentiation of human monocytes to dendritic cells and the activation of human cytotoxic T cells *in vitro*. Here we analyzed the immune modulation by lactate in a syngeneic mouse model *in vivo*. For this purpose we generated stable murine B16.SIY melanoma cell lines with low lactate secretion by means of *Ldha* short hairpin RNA technology. The growth of these tumor cells was followed in immunocompetent C57BL/6 mice, in immunodeficient Rag2$^{-/-}$ mice, lacking mature B and T cells and in immunodeficient Rag2$^{-/-}$ common-γ-chain$^{-/-}$ mice, additionally lacking NK cells. After s.c. application of 10^5 B16.SIY*Ldha-low* (lac ^{lo}) and control B16.SIY*Ldha-high* (lac ^{hi}) melanoma cells, a similar tumor growth occurred in Rag2$^{-/-}$ and Rag2$^{-/-}$ common-γ-chain$^{-/-}$ mice whereas tumor incidence and tumor growth of the B16.SIY*Ldha-low* (lac ^{lo}) clone was significantly delayed in immunocompetent C57BL/6 mice. This suggests that lactate modulates the T cell response in C57BL/6 mice. Therefore we determined the immune cell infiltrate in B16.SIY*Ldha-low* (lac ^{lo}) and control B16.SIY*Ldha-high* (lac ^{hi}) tumors and found no difference in tumor-infiltrating immune cells in Rag2$^{-/-}$ mice. In contrast, B16.SIY*Ldha-low* (lac ^{lo}) tumors exhibited significantly increased numbers of cytotoxic CD8$^+$ T cells and lowered numbers of myeloid CD11b$^+$Gr-1$^+$ suppressor cells in C57BL/6 mice.

We conclude that lactate is a potent immunoregulatory factor in the tumor environment. Therefore pharmacological inhibitors of tumor cell glycolysis could possibly not only target tumor cell growth directly but also support cancer immunotherapies.

DUSP4 Expression Is Associated with Microsatellite Instability in Colorectal Cancer (CRC) and Causes Enhanced Cell Growth

Benedikt Gröschl, Marcus Bettstetter, Christian Giedl, Matthias Woenckhaus, Tina Edmonston, Ferdinand Hofstädter, Wolfgang Dietmaier

DUSP4 (MKP-2), a member of the mitogen-activated protein kinase phosphatase (MKP) family and a potential tumor suppressor, negatively regulates the MAPKs (mitogen-activated protein kinases) ERK, p38 and JNK, which play a vital role in cancer development and progression. Using microarray analyses we found a remarkably frequent overexpression of DUSP4 in colorectal cancer (CRC) with high frequent microsatellite instability (MSI-H) compared to microsatellite stable (MSS) CRC. In this study we analyzed DUSP4 expression on mRNA level in 38 CRC (19 MSI-H and 19 MSS) compared to matched normal tissue as well as in CRC cell lines by *real-time* PCR. DUSP4 was overexpressed in all 19 MSI-H tumors and in 14 MSS tumors. Median expression levels in MSI-H tumors were significantly higher than in MSS-tumors. Consistently, MSI-H CRC cell lines showed 6.8-fold higher *DUSP4* mRNA levels than MSS cell lines. DUSP4 expression was not regulated by promoter methylation as no methylation was found by quantitative methylation analysis of the *DUSP4* promoter in CRC cell lines neither in tumor samples. In addition, no mutation could be detected in the protein coding regions of the DUSP4 Gene. Overexpression of recombinant DUSP4 in CRC cell lines caused upregulated expression of MAPK targets *CDC25A, CCND1, EGR1, FOS, MYC* and *CDKN1A* in HCT116 as well as downregulation of the mismatch repair gene *MSH2* in SW480. Furthermore, DUSP4 overexpression led to increased proliferation in CRC cell lines. Our findings suggest that DUSP4 acts as an important regulator of cell proliferation within the MAPK pathway and causes enhanced cell growth in MSI-H CRC.

A mutation in a mitochondrial protein causes renal Fanconi syndrome

Carsten Broeker, Markus Reichold, Christine Meese, Helga Schmidt, Enriko Klootwijk, Ralph Witzgall, Robert Kleta, Richard Warth

Introduction: Renal Fanconi syndrome is characterized by a reduced transport capacity of the proximal tubule and is accompanied by a renal loss of glucose, amino acids, bicarbonate, and phosphate among other metabolites. Prof. Kleta (University College, London) examined patients suffering from renal Fanconi syndrome. Genetic workup of these patients revealed a mutation in a gene coding for a mitochondrial protein, which we called FAP2.

Methods and results: Localization of FAP2 in human and mouse kidney was investigated on paraffin-embedded slices and cryosections, respectively. Expression was highest in the S1 segment of the proximal tubule of the kidney. To evaluate the effect of the mutation on cellular function, a stably transfected inducible cell line (LLC-PK1, originally derived from porcine proximal tubule cells) was generated overexpressing either wildtype FAP2 or the mutated protein. Localization of the protein was investigated via immunohistochemistry. Mitochondrial morphology was evaluated using electron microscopy. Confocal imaging of the cells showed mitochondrial localization of the protein in all stimulated cells. Mitochondria appeared slightly malformed in cells overexpressing wildtype protein. In mutant cells however, the mitochondria appeared elongated and filament-like. Electron microscopy showed engorged mitochondria with a diminished cristae count in wildtype cells. Cells expressing the mutant clearly showed elongated filament-like mitochondria with intramitochondrial deposits. High-resolution respirometry was conducted to evaluate mitochondrial function in stimulated cells. Mutant cells showed an increased O_2 consumption compared to cells overexpressing wildtype protein.

Discussion and future directions: Although this study is still at an early stage, preliminary results show significant changes in mitochondrial morphology and function in mutant cells. However, the underlying mechanism causing the disease has yet to be elucidated. We believe that impaired ATP generation in mitochondria leads to the reduced reabsorption in the proximal tubule. To this end further experiments are required, especially concerning the striking mitochondrial phenotype and its possible pathophysiological effects.

The role of the miRNA processing proteins Exportin 5 and Drosha in malignant melanoma

Corinna Ott, Prof. Dr. Anja Bosserhoff

MicroRNAs (miRNAs) are short non-coding RNAs that post-transcriptionally regulate gene expression via inhibition of translation or regulation of the stability of their complementary mRNA. Therefore, miRNAs control cellular processes and are key players in diseases such as cancer, including malignant melanoma.

After the transcription of the miRNA gene, the emerging pri-miRNA is processed by the microprocessor complex (Drosha and DGCR8) to a pre-miRNA. This pre-miRNA is then transported out of the nucleus by the karyopherin protein Exportin 5. In the cytoplasm, the pre-miRNA is further cleaved to its mature length. The functional strand of the miRNA is then loaded into the miRISC (miRNA-induced-silencing-complex), which can inhibit the target gene expression.

Previous studies already showed that altered miRNA expression can cause melanomagenesis and melanoma progression. Consequently, it is important to find out whether not only miRNAs but also miRNA processing enzymes (especially Drosha and Exportin 5) are deregulated in malignant melanoma. If a deregulation of these proteins can be observed, the aim is to find out to what extent altered miRNA processing affects melanoma development.

First, cultured cells as well as tissue samples from healthy skin, nevi, primary melanoma and metastases were comparatively examined by quantitative RT-PCR, Western blot, immunofluorescence and immunohistochemistry

In addition, we aim to find out how the up- or downregulation of these proteins influences the functional mechanisms of tumor development and -progression, such as proliferation, apoptosis, migration and invasion.

Moreover, the effect of deregulated Drosha and Exportin 5 expression on specific miRNAs will be examined.

Molecular mechanisms of early cancer dissemination in Balb-NeuT mice

Hedayatollah Hosseini, Mattias Maneck, Yves Hüsemann and Christoph A. Klein

A detailed analysis of spontaneous mouse models of breast cancer and of patients revealed that tumor cells spread much earlier than previously thought to distant sites. Furthermore, we found that the relative number of disseminating cancer cells is highest at early stages of tumorigenesis in Balb-NeuT mice, a transgenic spontaneous model of breast cancer. Mammary epithelial cells of Balb-NeuT mice displayed specific gene expression patterns at the stage of atypical hyperplasia (ADH), when dissemination of transformed is highest, that differed from advanced tumors and metastases. We therefore started to investigate whether this expression pattern is linked to the propensity to disseminate. First, we noted that many of the differentially expressed genes are regulated by the steroid hormones progesterone and testosterone. Then we used a surrogate signature, which can be induced by hormone stimulation, for the ADH-associated dissemination signature to further investigate mechanisms of early metastasis. This surrogate assay and western blot studies suggest that progesterone and androgen receptor activation cooperates with Her2 signaling to generate the ADH-associated dissemination signature. We will now search for the hormone responsive cells and the mechanisms of signal transduction. Once we have identified the cells that are particularly responsive to the dissemination-inducing signal, we will move on to identify factors that trigger transitions between migrating and stationary-proliferating states. This may identify relevant mechanisms of metastatic dissemination and colony formation at distant sites.

Enhanced renin cell recruitment in mice deficient for the cGMP-dependent protein kinase II

Birguel Kurt, Ilona Schwarzensteiner, Andrea Schramm, Jens Schlossmann and Armin Kurtz

Based on evidence that cyclic GMP activated protein kinase II (cGKII) inhibits renin secretion at the level of juxtaglomerular cells, this study aimed to address the role of cGKII in the integrative control of renin secretion and renin synthesis, in particular in the inhibitory feedback loops established by angiotensin-II, salt balance and blood pressure. Basal plasma renin concentrations (PRC) reflecting in vivo renin secretion were higher in cGKII knockout (-/-) than in wild-type (WT) mice but could be lowered by high salt intake in both genotypes. The response of plasma renin concentration to treatment with a low salt diet combined with the ACE inhibitor enalapril (LS+Ena) was increased in both genotypes, with a more distinct effect in the cGKII knockout. Treatment of mice with LS+Ena over three weeks led to a time dependent elevation of renin secretion and renin expression and to a concomitant decrease of blood pressure. Plasma renin concentration was clearly enhanced in cGKII -/- compared to wild-type controls. In parallel, renin mRNA abundance and kidney renin immunoreactivity were markedly enhanced in cGKII -/- relative to wild-type mice. Threedimensional reconstruction of intrarenal renin expression revealed an exaggerated recruitment of renin expressing cells in the media layer of preglomerular vessels, particularly in interlobular arteries. Mean blood pressure (MAP) measured via tail cuff method showed no significant differences between cGKII deficient and WT mice during treatment with LS+Ena and under normal conditions. As a consequence the relationship between blood pressure and renin secretion, renin gene expression as well as renin cell recruitment was clearly not altered in the absence of cGKII. Acute pressure regulation of stored renin as measured from isolated perfused kidneys was also not changed in the absence of cGKII.

We infer from our data that cGKII is not of major relevance for the negative feedback regulation of the renin system by high salt intake, nor for the negative feedback established by angiotensin-II (Ang-II) or blood pressure.

The mechanisms of compensatory renal hypertrophy - the role of B-type natriuretic peptide and the role of podocytes in GC-A-receptor-signaling pathway

Janina Staffel, Frank Schweda

Loss of functional kidney tissue results in an increase of weight and function of the remaining kidney tissue. Despite of its high clinical relevance the initial steps inducing this phenomenon, named compensatory renal hypertrophy (CRH), is largely unknown.

Our previous work has shown the guanylyl cyclase-A (GC-A) to play an important role in the development of CRH. GCA is the common receptor for natriuretic peptides ANP (Atrial natriuretic peptide) and BNP (B-type natriuretic peptide), that both induce natriuresis, diuresis and an increase in glomerular filtration rate (GFR). Recent research results suggest that in CRH BNP rather than ANP activates GCA and hereby leads to the initial increase of kidney function and growth. In the first part of my project the precise functional role of BNP will be investigated by generating a BNP-knockout-mouse. After inducing CRH by unilateral nephrectomy, different clinical parameters such as urine volume, electrolyte concentration and GFR as well as kidney weight and protein/DNA-ratio of the remaining kidney will be determined at various time points post surgery.

As mentioned before it is known that ANP and BNP cause an increase in GFR by activating GC-A in the kidney. The receptor is highly expressed in many different kidney structures, such as afferent and efferent arterioles, glomerular capillaries, mesangial cells, tubules and also in podocytes. We speculate that podocytes are involved in the regulation of GFR by their contractile capacity. These cells surround the glomerular capillaries, form a part of the glomerular filtration barrier on the one hand and support the capillaries against the high capillary pressure on the other hand. A dilatation should cause an expansion of capillary wall which causes an increase in filtration surface in turn and thereby an increase in GFR. By generating a podocyte specific GC-A-knockout-mouse (GC-A flox/PodocinCre+) this hypothesis will be tested in the second part of my project.

Role of MTA in malignat melanoma

Katharina Limm, Corinna Ott, Susanne Wallner, Anja-Katrin Bosserhoff

The enzyme Methylthioadenosine phosphorylase (MTAP) is expressed ubiquitously in normal cells. Its corresponding gene is localized in the chromosomal region 9p21, near several tumour suppressor genes (p15, p16, p14ARF). The function of MTAP is the conversion of 5'-desoxy-5'-methylthioadenosine (MTA) to adenine and 5'-methylthioribose. In several types of tumors, such as melanoma and hepatocellular carcinoma, the expression of MTAP is strongly reduced. Due to this, MTA accumulates intracellularly as well as extracellularly. We could show that MTA has an impact on cellular signaling, proliferation and migration. So far, the molecular mechanism of this regulation is still unkown. Treatment with a defined MTA concentration leads to a cellular response. Analysing these will help to unravel these molecular mechanisms.

One focus is the search for putative receptors relevant for the mode of action of this small molecule. Having a similar structure to adenosine, the adenosine receptors (ADORAs) may play an important role in MTA signaling. We found in our melanoma cell lines that one of four known types of receptors (ADORA1, A2A, A2B and A3) is stronger expressed (mRNA level) than the others. Extracellular stimulation of these receptors can be monitored via impedance measurement. This is possible through the detection of G-Protein coupled reactions with the xCelligence-System (Roche).

Treatment with MTA also leads to an intracellular signaling. One significant effect is the increased AP1 activity. This could be shown in AP1-luciferase activity assays by transient transfection with an AP1-luciferase construct and was also confirmed by EMSA (Electrophoretic Mobility Shift Assay). Another significant impact on cellular pathways is the inhibitory effect on PRMTs (protein arginine methyltransferases). Further, it could be shown that protein methylation has an effect on ERK signaling in cells. Therefore we analyzed the impact of MTA on symmetric and asymmetric protein methylation and ERK phosphorylation in western blots.

These results indicate that the secretion of MTA has an impact on intracellular processes. More target genes, which are affected by MTA, still have to be identified to get a total view on all of its effects on cellular.

The influence of type XVI Collagen on cell growth and invasion in oral squamous cell carcinoma

Konstanze Bedal, Richard Bauer, Susanne Grässel, Torsten Reichert

Type XVI collagen (Col XVI) belongs to the family of FACIT collagens (*fibril associated collagen with interrupted triple helix*). In the oral mucosa, it is localized at the dermal epidermal junction zone. There, Col XVI is important for the integrity of the basal membrane and the cross linking of elastic fibrils.

In preliminary works, we observed an overexpression of Col XVI and in oral squamous cell carcinoma (OSCC) patients. Overexpression of Col XVI in the human OSCC cell line PCI13 disclosed a positive impact on cell proliferation and invasion. On protein level we detected a strong influence of Col XVI on the activity of integrin beta 1 (ITGB1) and an increase of the integrin-associated protein Kindlin-1 in Col XVI overexpressing PCI13 cells. Moreover, the induction of Col XVI expression caused an increased expression of MMP-9 on mRNA and protein level.

One of the aims of this thesis is to investigate the induction of the activation of the proliferation signalling cascade in malignant oral keratinocytes. We demonstrate that Kindlin-1 interacts with the integrin-linked kinase (ILK) in Col XVI overexpressing PCI 13 Cells in contrast to mock control cells. Col XVI overexpressing PCI13 cells show increased levels of phosphorylated and therefore activated ILK. In these cells we observe higher amounts of phosphorylated protein kinase B (Akt/PKB) which is known to activate ILK by phosphorylation.

Moreover, MMP-9 promoter studies indicate three potential transcription factor (TF) binding sites, AP-1, WT-1 and FoxP3, which seems to connect ILK activation with MMP-9 gene expression.

In addition, we will analyze the modulation of signalling pathways by Col XVI in normal oral keratinocytes.

Furthermore, we showed that MMP-9 was able to truncate recombinant Col XVI. Therefore, it should be analyzed if the found fragments of Col XVI have a tissue homeostatic function like other known collagen fragments such as Endostatin or Tumstatin.

TMEM16 proteins partially produce Volume-activated Chloride Current

Lalida Sirianant, Jiraporn Ousingsawat, Rainer Schreiber, Karl Kunzelmann

Volume-regulated anion channel (VRAC) is an unknown molecular identity chloride channel that is responsible for regulatory volume decrease (RVD) in many cell types. When cells are exposed to hypotonic solution, RVD process conducts by inducing chloride efflux through VRAC. We have been shown that TMEM16 proteins have potential role in cell volume regulation. Here we found that in HEK293 cells, 155 mOsm hypotonic solution activated Cl^- current but not K^+ current. This volume-activated chloride current was not inhibited by 50 µM NPPB, a blocker of VRAC. Interestingly, this volume stimulated current became sensitive to NPPB when extracellular Ca^{2+} concentration ($[Ca^{2+}]_e$) was reduced from 1 mM to 0.1 µM. These results indicated a function of VRAC at low $[Ca^{2+}]_e$. In addition the cell swelling activated Cl^- current at 0.1 µM $[Ca^{2+}]_e$ was significantly smaller compared to 1mM $[Ca^{2+}]_e$. These data suggested that at physiological $[Ca^{2+}]_e$ (1 mM) TMEM16 proteins and VRAC were involved in RVD in HEK293 cells. We further investigated the mechanism of cell swelling stimulated VRAC by using the Ca^{2+} chelator, BAPTA-AM. Cells pre-incubated with BAPTA-AM did not longer response to hypotonic solution indicating that Ca^{2+} is necessary for function of VRAC. This study suggests that during the cell swelling, TMEM16 proteins are partly produce volume-activated chloride current and Ca^{2+} is required in order to produce volume-regulated chloride current.

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Role of MIA signaling in malignant melanoma

Lena Honold, Anja K Bosserhoff

Malignant melanoma is a skin tumor which arises from the pigment-producing cells of the skin, the melanocytes. If a malignant melanoma is detected and removed by excision at an early stage, a full recovery is possible in about 80% of all cases. Since it a metastasizes early, already at a small primary tumor the survival rate at metastatic, advanced stages decreases to an average of six months. In summary, melanoma is the most common lethal skin disease worldwide with rising number of new cases.

It is known that the protein *melanoma inhibitory activity* (MIA) has an influence on migration and invasion of melanoma cells, and thus plays a role in the metastasis.

MIA, together with three other homologous members (MIA2, OTOR and TANGO), belongs to the MIA protein family. In contrast to normal melanocytes, melanoma cells secrete large amounts of the protein MIA which interacts with components of the extracellular matrix such as laminin or fibronectin. MIA then inhibits binding of these cells to the extracellular matrix and causes detachment of individual cells from the cell cluster.

Previous studies indicate that MIA is also involved in protein regulation via binding to integrins leading to a reduced activity of the protein kinases ERK1/2 in melanoma cell lines. However, so far it is not known which signaling pathways mediate MIA-dependent gene regulation. To analyze potential candidate pathways, reporter gene assays for AP-1, NF_kB, cre, SRF, SRE were performed after addition of recombinant MIA. Furthermore, the effects of different MIA concentrations on attachment and proliferation of melanoma cells were investigated by real-time cellular analysis (RTCA).

In the experimental model of leishmaniasis myeloid-derived suppressor cell functions and interaction with parasites differs between C57BL/6 and BALB/c

Maximilian Schmid, Nicole Zimara and Uwe Ritter

Myeloid-derived suppressor cells (MDSC) represent a heterogenous cell population characterized by an immature differentiation state and immunosuppressive potential. According to the surface molecules Ly6G and Ly6C, MDSCs can be further dissected into monocytic (MO-MDSC, $CD11b^{+}/Ly6G^{-}/Ly6C^{high}$) and polymorphonucleated myeloid-derived suppressor cells (PMN-MDSC, $CD11b^{+}/Ly6G^{+}/Ly6C^{int}$). Myeloid cells with suppressive capacities were described for the first time in patients suffering from cancer or autoimmune diseases. However, little is known about the impact of MDSCs in innate and adaptive immunity against parasitic infections. Thus, we used the experimental model of leishmaniasis to assess the potential role of MDSCs in modulating the immune response against *Leishmania (L.) major*. Two different mouse strains were included in the study: i) C57BL/6, which are known to cure the parasite infection by T_H1-mediated immune response ii) BALB/c mice, incapable of eliminating the parasites due to a T_H2-driven humoral immune response.

We were able to show that the total number of MDSCs increases in the blood, skin-draining lymph nodes, spleen and in the footpad of infected mice. An interesting observation was the reduced number of monocytic MDSCs in all analyzed organs in BALB/c mice compared to C57BL/6 indicating a severe defect in generating this cell population. We generated *in vitro* MDSCs to investigate parasite-suppressor cell interaction in more detail. Here we could demonstrate a higher parasite incorporation in BALB/c MDSCs than in C57BL/6 cells. Furthermore, *in vitro* generated MDSCs were able to inhibit Leishmania-specific T cell proliferation. Infected MDSCs generated from BALB/c mice lost their suppressive effect whereas C57BL/6 MDSCs maintained their suppressive potential.

In conclusion we showed that both the i) infiltration of and ii) the interaction with MDSCs differs between resistant and susceptible mouse strains after infection with *L. major* parasites. Further studies will be necessary to characterize the impact of MDSC on the course of leishmaniasis.

Role of CYLD in early melanoma development

Miriam deJel, Silke Kuphal, Susanne Schiffner, Anja K.Bosserhoff

The tumor suppressor function of CYLD was first identified through genetic analysis of familiar cylindromatosis, which is an autosomal-dominant disorder characterized by a predisposition to benign skin tumors. Since then, mutation and depletion of CYLD has been associated with tumor development and progression of other cancers, such as breast cancer, renal cell carcinoma, colon cancer and malignant melanoma. The last is a malignant tumor of pigment-producing cells. It is the most aggressive form of skin cancer, with incidence rates increasing annually. In melanoma, expression of *CYLD* is down-regulated in consequence of increased activation of the transcription factor snail1, resulting in increased proliferation and invasion of melanoma cells.

Here, we characterize CYLD with respect to its tumor suppressor function in melanoma. *In vivo* analyses of a CYLD knockout mouse model show that *Cyld* deficient mice evolve melanoma significantly earlier and have an accelerated tumor growth compared to the control group. Moreover, *in vitro* studies with human melanoma cell lines confirm that CYLD has a repressive effect on colony size.

CYLD exhibits a catalytic domain which allows deubiquitination of proteins. Through hydrolyzation of ubiquitin molecules from TRAF2, NEMO and Bcl-3, CYLD inhibits both the classical and the alternative NF-κB pathways, impeding tumor progression. In line with this, we can demonstrate that CYLD reduces NF-κB activity and induces apoptosis in human melanoma cells.

In addition, qRT-PCR analyses exhibit that CYLD represses the expression of several stem cell markers in human melanoma cells and thus inhibits de-differentiation of tumor cells. Moreover, murine tumor samples of *Cyld*$^{-/-}$ mice display a higher expression of stem cell markers than tumors of Wild type mice. Therefore, these results indicate a new tumor suppressor mechanism of CYLD.

Molecular function of the liver protein MIA2

Mona Solanki, Jacqueline Schlegel, Anja K. Bosserhoff

The liver is the largest organ inside the human body and is responsible for various processes regarding detoxification, protein synthesis and metabolic pathways. Liver diseases like fibrosis or cirrhosis can be caused by different factors such as drugs, alcohol, diabetes or viruses. Ongoing damage can lead to hepatocellular carcinoma, the fifth most common cancer in the world.

Melanoma inhibitory activity 2 (MIA2) is an approximately 60 kDa protein which is expressed specifically in the liver. It is secreted by the hepatocytes and controlled by an N-terminal signal sequence which causes its transport into the extracellular space. MIA2 belongs to the MIA gene family sharing similarities in homology, structure and genomic organization with the other members MIA, OTOR and TANGO.

MIA2 mRNA is highly overexpressed in patients with liver diseases, leading to the conclusion that MIA2 expression responds to liver damage. Interestingly, the protein expression in hepatocellular carcinoma (HCC) is down-regulated. Based on these findings, MIA2 acts as a tumor suppressor and therefore might have therapeutic potential. For that reason it is important to understand its molecular function in detail.

Regulation of MIA2 protein expression is investigated in this work, focusing on the potential influence of miRNAs. Here, putative miRNA binding sites were defined *in silico* and then mutated in a MIA2 expression construct. Afterwards, MelHo and HepG2 cells were transiently transfected with these constructs. The MIA2 expression was then analyzed via SDS-PAGE, Western Blot and qRT-PCR.

Furthermore, it is known that MIA2 has an SH3 domain which mediates protein-protein interactions. To find out more about potential interaction partners of MIA2, a yeast-two-hybrid assay was performed. In this screening, FAT1, an integral membrane protein of the cadherin family emerged to possibly interact with MIA2. MIA2-FAT1 interaction will be further analyzed by e.g. co-immunoprecipitation and other techniques.

Characterization of bactericidal/permeability increasing protein expression and function based on newly developed transgenic mouse models.

Nicole Bezold, Joachim Gläsner, André Gessner

Bactericidal/permeability increasing protein (BPI) belongs to the highly conserved family of lipid-transfer-proteins. Based on their common BPI-domain, they are also referred to as BPI-family. Human BPI is synthesized early during myelopoiesis by granulocyte precursor cells of the bone marrow and is mainly stored in primary granules of neutrophil granulocytes and to a lesser extent in eosinophil granulocytes. Beyond that, BPI could be localized on the surface of neutrophils and monocytes due to degranulation of adjacent neutrophils. More recently, expression of BPI could be shown in mucosal epithelial cells of the airways, intestinal tract, genitourinary system and the lacrimal duct as well as in fibroblasts. Its molecular mass is approximately 55-60 kDa and X-ray crystallography revealed a boomerang-shaped structure built up of two functional domains, characteristic for the BPI-family. The cationic N-terminal domain is responsible for the entire bactericidal and bacteriostatic impact on gram-negative bacteria together with binding to lipopolysaccharide (LPS). Compared to other LPS-binding proteins, BPI displays a higher affinity for the lipid A region of LPS. Thereby, it efficiently neutralizes endotoxin-mediated pro-inflammatory responses. Binding to bacteria increases membrane permeability, interrupts cell division and induces cell lysis. The C-terminal domain causes opsonization by the recruitment of complement factors resulting in an enhanced phagocytosis. The importance of BPI in the innate immunity is highlighted by the known polymorphisms in the BPI-Gen. For instance, the chronic inflammatory bowel disease Morbus Crohn is associated with one of these polymorphisms. Patients show an increased expression of BPI. In patients with sepsis the expression of BPI positively correlates with the degree of the clinical course. Despite the initial description of BPI in the late 1970s there are no publications describing the role of endogenously produced BPI in infections. The assigned purpose of this project is a more detailed insight into the functions of this highly conserved defense protein during infectious diseases based on experiments with newly designed BPI-knockout and -transgenic (-humanized) mouse models.

Anoctamin1 expression in carcinoma cells

Podchanart Wanitchakool, Luisa Wolf, Andreas Gaumann, Rainer Schreiber, Karl Kunzelmann

Anoctamin1 (Ano1, TMEM16A) is Ca^{2+}-activated Cl^- channel. Ano1 is highly up-regulated in gastrointestinal stromal tumors (GIST), oral squamous cell carcinomas and human neck squamous cell carcinomas. Here we demonstrate that expression of Ano1 is regulated by the tumor suppressor adenomatosis polyposis coli (APC). Mutation of the APC protein ($APC^{min/+}$) in a cancer mouse model causes development of polyps in small and large intestine leading premature death. The mRNA and protein expression of Ano1 were reduced in colonic crypt cells isolated from $APC^{min/+}$ mice compared to wild type animals. Our recent study showed that the mTOR inhibitor rapamycin markedly inhibited the APC mutation related polyposis. We found that the treatment of $APC^{min/+}$ mice with rapamycin increased the expression of Ano1 suggesting an anti-proliferative function of Ano1. We further investigated the expression level of Ano1 in human colonic carcinoma HT_{29} cells. Treatment of HT_{29} cells with valproic acid increase expression of ANO1. Down regulation of APC protein expression using siRNA in valproic acid treated HT_{29} cells caused reduction of the mRNA level of Ano1. This decrease in Ano1 expression was attenuated by FH535, an antagonist of β-catenin signaling. These data suggest that ANO1 is regulated by the Wnt signaling pathway and may prevent polyp formation. The specific function of ANO1 in this model will be investigated in future studies.

The role of histone modifications in the pathogenesis of glioblastoma and their relevance as a potential novel treatment option.

Sabine Hoja, Markus J. Riemenschneider

Histone modifications, such as acetylation or methylation, influence the overall structure of chromatin and can thereby positively or negatively regulate gene expression. The state of chromatin can change from euchromatin, the lightly packed form that is often transcriptionally active, to the more tightly packed heterochromatin that is primarily not transcribed. In gliomas, the most common human primary brain tumor with high potential for malignant progression and invasion, histone modifications have been shown to modify gene transcription. They can be investigated via chromatin immunoprecipitation (ChIP), a method to study protein-DNA-interactions. Proteins are isolated with antibodies that are specific to a certain feature of the chromatin, e.g. a particular histone modification, and the DNA bound to these proteins can be used for assessment of gene promoters bound to these histones.

In re-establishing an *in vitro* ChIP protocol in our lab, different glioblastoma cell lines were treated with Trichostatin A, a histone deacetylase inhibitor that leads to the stabilization of euchromatin. ChIP analyses were performed with antibodies against acetylated histones H3 and H4, histone modifications that are associated with euchromatin. Efficacy of TSA treatment was controlled by real-time RT-PCR with primers for *RRP22* (a gene that in our lab had been previously shown to be transcriptionally repressed by heterochromatinization), *p21* (known to be inactivated by histone modifications in human gliomas) and *GAPDH* (a house-keeping gene residing in a euchromatic stage).

The next step of the project will now be to repeat the above ChIP protocol after treatment of glioma cells with valproic acid, another histone deacetylase inhibitor that in gliomas has been shown to enhance the effects of standard temozolomide chemotherapy. ChIP-Seq analysis will then be used to pinpoint the exact mechanisms of the agent’s antitumor activity and to uncover novel epigenetic chromatin-based mechanisms of importance to gliomagenesis and chemotherapy response.

DLX3 Regulates Apoptosis and the Osteogenic Differentiation of Human Dental Follicle Precursor Cells

Sandra D. Viale Bouroncle

Objectives: The transcription factor DLX3 plays a decisive role in bone development of vertebrates. In neural-crest derived stem cells from the dental follicle (DFCs), DLX3 is differentially expressed during osteogenic differentiation. However, other osteogenic transcription factors such as RUNX2 are not highly induced, suggesting a decisive role of DLX3 in the differentiation of DFCs. Moreover, a microarray analysis after DLX3 overexpression showed that marker genes for proliferation, apoptosis, and osteogenic differentiation were significantly regulated. This study investigated therefore the influence of DLX3 on proliferation, apoptosis, and osteogenic differentiation in DFCs.

Methods: DLX3 was overexpressed or silenced in DFCs, using the pCMV-V5-DLX3 plasmid or a DLX3 specific siRNA. Cell proliferation was compared using a WST-1 assay; apoptosis with Annexin V-FITC/PI; and osteogenic differentiation with alkaline phosphatase activity, alizarin red staining and expression of osteogenic markers.

Results: After DLX3 overexpression, cell viability is directly influenced by the expression of DLX3, for example, the amount of apoptotic cells in DFCs was increased after DLX3 silencing. DLX3 stimulates the osteogenic differentiation of DFCs and regulates the BMP-SMAD1 pathway. BMP2 did highly induce DLX3 and reverse the inhibitory effect of DLX3 silencing in osteogenic differentiation. However, after DLX3 overexpression in DFCs, a high-dose BMP2 supplementation did not improve the expression of DLX3 and the osteogenic differentiation.

Conclusion: DLX3 influences cell viability and regulates osteogenic differentiation of DFCs via a BMP2-dependent pathway and a feedback control.

ALR – a novel and auspicious target for NASH treatment?

Susannah Spieker, Thomas S. Weiss

Nonalcoholic fatty liver disease (NAFLD) is an important cause of liver disease in adults and even the most common liver disease in children. The prevalence of NAFLD is increasing worldwide due to growing obesity. NAFLD's clinical spectrum ranges from simple fat accumulation in the liver (steatosis) to an inflammatory and fibrotic stage termed nonalcoholic steatohepatitis (NASH). Furthermore, NASH may progress to severe fibrosis, cirrhosis, hepatocellular carcinoma (HCC) and may without treatment ultimately lead to liver failure. At the moment, there exists no effective treatment strategy except the compensation of risk factors, like e.g. the loss of weight for obese people. The protein augmenter of liver regeneration (ALR) seems to be a promising target for the treatment of this disease because it exhibits hepatoprotective properties and promotes hepatocyte proliferation. The overall aim of this study is to investigate the regulation, expression and functional role of ALR in NASH. In particular, *in vitro* and *in vivo* NASH models will be used to elucidate, dependent on different isoforms of ALR, its potential protective role under conditions of oxidative stress and inflammation. First, we prepared both isoforms of ALR (short, sfALR; long, lfALR) as recombinant proteins expressed in *E. coli* and generated hepatoma cell lines (HepG2, Huh-7), which stably express ALR (sfALR, lfALR). Second, we established *in vitro* NASH/steatose models by feeding hepatoma cells and primary human hepatocytes (PHH) with free fatty acids leading to enhanced triglyceride storage. Preliminary data confirm the proposed localization of sfALR and lfALR as cytsolic and mitochondrial, respectively. Significantly lower fatty acid induced cell toxicity was found in ALR (sfALR, lfALR) expressing cells compared to wildtype cells. Future experiments will focus on expression profiles of wildtype and ALR-transfected cells under conditions of NASH regarding genes involved in apoptosis, oxidative cell activity, inflammation and fibrosis. Furthermore, impact of ALR (released by hepatocytes) on hepatic stellate cells, which are mainly responsible for collagen synthesis and fibrosis progression, will also be analyzed. Finally, an *in vivo* approach will be performed to evaluate data obtained from *in vitro* experiments.

THE SPATIAL ENERGY EXPENDITURE CONFIGURATION AND POSSIBLE APPLICATIONS IN AN EXPERIMENTAL MODEL OF ARTHRITIS

Susanne Klatt, Rainer H. Straub

Background: An autoimmune response with differentiation and proliferation of immune cells and the subsequent tissue-directed inflammatory process in the symptomatic phase of the disease are very energy-demanding. As recent calculations demonstrate, the activated immune system needs approximately 20% of the basal metabolic rate. Thus, energy regulation and cellular bioenergetics are of outstanding importance to serve a stimulated immune system. During inflammation, particularly during the chronic process of inflammation in long standing inflammatory diseases like rheumatoid arthritis, a reallocation of energy-rich fuels to the activated immune system is necessary in order to nourish the inflammatory process. Energy consumption and, thus, ATP generation can be measured by studying the consumption of oxygen.

The energy expenditure in different organs at different time points has never been investigated during immunization (the symptomatic phase of the disease). We want to find out if, and how the energy expenditure in different organs changes during the course of experimental arthritis.

Methods and Results: A new technique termed “spatial energy expenditure configuration (SEEC)” was developed to demonstrate bodily areas of high energy demand. SEEC is based on removal of tissue during the course of arthritis, and subsequent determination of oxygen consumption. For that purpose, small weighed pieces of the respective organ with a size of 4 mm are placed in 24-well multidishes with integrated oxygen sensors, which allows for non-invasive detection of oxygen consumption *in vitro*. SEEC was established in healthy control animals, arthritic animals and animals that underwent prior sympathectomy. The model of type II collagen arthritis in DBA/1 mice is used in order to develop an arthritic-specific SEEC. We determined the oxygen consumption in spleen, thymus, draining lymph nodes, liver, kidney, brain and knee joints during the course of experimental arthritis for 70 days. The values are given in µmol O_2/l/h and refer to 4 mm sized pieces as percentage of mouse weight.

Concerning the draining lymphoid nodes, we were able to observe a marked increase in oxygen consumption (200 %) during the course of arthritis. Other investigated organs like liver or kidney decrease their oxygen consumption (control vs. arthritic animals).

Conclusions: The SEEC technique enables us to identify locations of high energy demand that are involved in the initiation and continuation of the autoimmune process in an animal model of arthritis. We identified the draining lymph nodes as target organ of the sympathetic nervous system, which will be further investigated. The technique will be applied to other chronic inflammatory disease models in order to detect further participating organs.

Talks Part II

Characterization of the anti-inflammatory impact of molecular fragments derived from the adipokine CTRP-3 in adipocytes and monocytes

Andreas Schmid Talk 6

Creation and Characterization of *Pkd2* Knock-in Mice

Denise Schmied Talk 7

The impact of the basolateral potassium channel KCNJ10 for salt resorption in the distal tubule of the kidney

Maria Ripper Talk 8

The physiological and pathophysiological relevance of the inwardly rectifying potassium channel Kir5.1 (KCNJ16) in the kidney

Evelyn Humberg Talk 9

Characterization of the anti-inflammatory impact of molecular fragments derived from the adipokine CTRP-3 in adipocytes and monocytes

Andreas Schmid, Andrea Kopp, Andreas Schäffler, Christa Büchler

Introduction

C1q/TNF-related protein-3 (CTRP-3) is a newly discovered adipokine which is strongly up-regulated during adipocyte differentiation and plays an important role in adipocyte physiology and metabolism. CTRP-3 has also been shown to act as an anti-inflammatory lipopolysaccharide (LPS) antagonist by decreasing the secretion of pro-inflammatory cytokines, e.g. interleukine 6 (IL-6) *in vitro* in adipocytes and monocytes. The molecular mechanisms underlying these effects in detail remain unclear so far. In this context, the potentially differential effects of the globular and the collagen-like domain of CTRP-3 are still to be investigated in comparison to the observed effects exerted by the full length protein.

Methods

Recombinant full-length CTRP-3 and its globular domain (gCTRP-3) were expressed in HighFive cells and in HEK293 cells, the secreted proteins were extracted from the cell culture supernatant and purified by affinity chromatography.

For stimulation experiments, differentiated 3T3-L1 and monocyte-like THP-1 cells were used.

Release of pro-inflammatory cytokines IL-6, monocyte chemotactic protein 1 (MCP-1), resistin and TNF-alpha was induced by addition of LPS or free fatty acids to the medium and was measured with ELISA techniques. Costimulation experiments with these stimuli plus CTRP-3 were performed to analyse its impact on the secretion of pro-inflammatory cytokines.

RT-PCR based mRNA analyses were performed to investigate regulation of pro-inflammatory factors like IL-6 and MCP-1 on transcription level.

CTRP-3 was also tested in an animal model of LPS-induced SIRS (C57BL/6 mice) and the expression of pro-inflammatory cytokines was analysed in blood serum and in adipose tissue.

Results

Full-length CTRP-3 was shown to significantly decrease the amount of secreted IL-6, MCP-1 and resistin after costimulation with LPS in differentiated 3T3-L1 cells. In THP-1 cells, treatment with CTRP-3 resulted in reduced IL-6 release. The anti-inflammatory effects observed in cultured cells were confirmed *in vivo*. Our first results for the globular domain have still to be verified.

Conclusion and outlook

We observe a strong and significant anti-inflammatory property of CTRP-3 which might be mediated by its globular domain. Further experiments will have to determine the molecular components and mechanisms responsible for the observed effects.

Creation and Characterization of *Pkd2* Knock-in Mice

Denise Schmied, Karin Babinger, Ralph Witzgall

Introduction: Autosomal-dominant polycystic kidney disease (ADPKD) is a monogenetic disorder with a prevalence of 1:1000. ADPKD is responsible for about 10% of all cases of end-stage renal disease. Approximately 50% of the patients develop chronic kidney failure at the age of 60. ADPKD is caused by mutations in *PKD1* and *PKD2*, 85% of patients carry mutations in the *PKD1* gene. Mutations in *PKD2* lead to a less progressive disease than mutations in *PKD1*. *PKD1* and *PKD2* encode the proteins polycystin-1 and polycystin-2. To date the function of polycystin-1 is not well understood. Polycystin-2 is an integral membrane protein of 968 amino acids in human, it functions as a non-selective cation channel.

Goal and methods: The aim of my work is to examine the development of ADPKD in two knock-in mouse models which were generated previously. In the first mouse model, *Pkd2*^{PoreL1}, the pore-forming segment of polycystin-2 was replaced by that of polycystin-2L1. In the second model, *Pkd2*^{L701}, polycystin-2 lacks its C-terminus and the protein has just 701 instead of 966 amino acids. So, the new approach of this work is to exchange specific gene sequences instead of creating a null mutant. Thus, the function of polycystin-2 can be investigated in more detail.

Results: To investigate the influence of the genetic background caused by modifier genes, the two mouse models were generated with two different strains, C57BL/6 and 129Sv. We analyzed from both mouse models at different ages the kidney, liver and pancreas after PFA fixation and searched for tissue alterations. In homozygous *Pkd2*^{PoreL1} mice we could find slight alterations in the kidney compared to wild-type mice. Homozygous *Pkd2*^{L701} mice show rapid of cyst formation in the kidneys. Additionally the tissue of these mouse models will be used to determine the localization of the mutant proteins in the kidney by immunostaining. Likewise, it is known that the calcium flow is changed in modified polycystin-2 proteins. Therefore the channel activity of the pore region should be analyzed. Therefore, we are establishing a polycystin-2 protein which will be fused to a calcium-sensitive fluorescence marker to analyze the calcium flow through the pore region. Furthermore it is planned to analyze the conductivity and the opening state of the pore region by patch-clamp. To determine which amino acids of the pore region are embedded into the lipid bilayer cysteine-scanning mutagenesis will be done. These results should lead us to a better understanding of the pore region of polycystin-2.

The impact of the basolateral potassium channel KCNJ10 for salt resorption in the distal tubule of the kidney

Maria Ripper, Markus Reichold, Sascha Bandulik, Ines Tegtmeier, Richard Warth

Introduction

Mutations in the K^+-channel KCNJ10 (Kir4.1) are causative for the autosomal recessive EAST syndrome (also referred to as SeSAME syndrome) which is characterized by **e**pilepsy, **a**taxia, **s**ensorineural deafness and a salt-wasting **t**ubulopathy. The renal salt-wasting pathology is caused by transport defects in the distal convoluted tubule and the connecting tubules where KCNJ10 plays a pivotal role as a basolateral K^+-channel. While in the early parts of the nephron the vast majority of the filtrate is reabsorbed, in post-macula densa segments fine tuning of the urinary composition takes place. All mutations in KCNJ10 which have been found in EAST patients so far lead to a partial or even a total loss of channel function. As a consequence, transport processes in the distal convoluted tubules and connecting tubules are impaired.

Methods/Results

Total KCNJ10 knockout mice showed failure to thrive and died within the first week after birth. To investigate the renal phenotype of adult KCNJ10$^{-/-}$ animals we generated two different mouse lines with either kidney-specific (Ksp-Cre) or Doxycycline-inducible kidney-specific (Pax8-TetOCre) inactivation of KCNJ10. Both Cre-mouse lines were crossed with a Kir4.1-floxed mouse line and in the offspring the gene of interest (Kir4.1) was deleted via recombination at the Kir4.1-flanking loxP sites. Using realtime PCR the Kir4.1-mRNA-expression was tested. The kidney-specific mouse line showed 75% reduction of Kir4.1 expression, whereas the inducible kidney-specific mouse line revealed about 90%. To further investigate the expression rate of cre recombinase, a cre reporter strain (tdTomato/tdEGFP) was crossed with each mouse line to confirm the cells with cre recombinase activity and inactivity either via red or green fluorescence.

Discussion/Perspective

In further experiments we want to characterize the two mouse lines by functional measurements of the kidney, telemetric blood pressure measurements, electron microscopic investigations of the distal tubule regarding morphological changes and finally an electrophysiological characterization of isolated distal tubules.

The physiological and pathophysiological relevance of the inwardly rectifying potassium channel Kir5.1 (KCNJ16) in the kidney

Evelyn Humberg, Markus Reichold, Sascha Bandulik, Ines Tegtmeier, Ralph Witzgall, Christine Meese, Helga Schmidt, Richard Warth

Introduction

Mutations of the inwardly rectifying K^+ channel subunit Kir4.1 (KCNJ10) are causative for an autosomal recessive disease, which is characterized by **e**pilepsy, **a**taxia, **s**ensorineural deafness, and a salt-wasting renal **t**ubulopathy (**EAST** or SeSAME syndrome). The Kir4.1 subunit is believed to form heteromeric K^+ channels with Kir5.1 in the kidney. Therefore we are investigating the physiological and pathophysiological relevance of the Kir5.1 subunit and the Kir4.1/Kir5.1 heteromeric channel for kidney function.

Results and Methods

To study the role of the Kir5.1 subunit we are using conventional Kir5.1 knockout (*Kir5.1*$^{-/-}$) mice in a SV129 background. Under normal diet *Kir5.1*$^{-/-}$ mice appeared normal without any growth retardation or apparent morphological abnormalities. Previously, we observed that the mutated *Kir4.1* subunit led to altered morphology of distal convoluted tubule (DCT) cells: the number of infoldings of the basolateral membrane as well as the number of mitochondria was reduced. Interestingly, DCT cells of *Kir5.1*$^{-/-}$ mice did not show any morphological abnormalities. However, electrolyte measurements of plasma and urine were indicative for a disturbed electrolyte balance in *Kir5.1*$^{-/-}$ mice. Especially during early postnatal development, *Kir5.1*$^{-/-}$ mice suffered from loss of electrolytes via the urine. Glomerular filtration rate and blood pressure of adult *Kir5.1*$^{-/-}$ mice, however, were not different from those of *Kir5.1*$^{+/+}$ mice.

Discussion and Perspective

The Kir5.1 potassium channel subunit seems to be important for the control of electrolyte reabsorption in the DCT, especially during early postnatal live. The next step is to investigate the influence of different diets on urinary excretion of electrolytes, blood pressure and plasma electrolytes. On the cellular level, we want to find out if the missing Kir5.1 subunit has an effect on membrane voltage, potassium current, and pH dependency of DCT cells.

Talks Part III

Superficial Nephrons in BALB/c and C57BL/6 Mice Facilitate in vivo Multiphoton Microscopy of the Kidney
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Increased KGF Expression Promotes Fibroblast Activation in a Double Paracrine Manner Resulting in Cutaneous Fibrosis.
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Superficial Nephrons in BALB/c and C57BL/6 Mice Facilitate in vivo Multiphoton Microscopy of the Kidney

Ina Schießl, Hayo Castrop

Multiphoton microscopy (MPM) offers a unique approach for addressing both the function and structure of an organ in near-real time in the live animal. The method however is limited by the tissue-specific penetration depth of the excitation laser. In the kidney, structures in the range of 100 µm from the surface are accessible for MPM. This limitation of MPM aggravates the investigation of the function of structures located deeper in the renal cortex, like the glomerulus and the juxtaglomerular apparatus. In view of the relevance of gene-targeted mice for investigating the function of these structures, we aimed to identify a mouse strain with a high percentage of superficially located glomeruli. The mean distance of the 30 most superficial glomeruli from the kidney surface was determined in 10 commonly used mouse strains. The mean depth of glomeruli was 118.4±3.4, 123.0±2.7, 133.7±3.0, 132.3±2.6, 141.0±4.0, 145.3±4.3, 148.9±4.2, 151.6±2 .7, 167.7±3.9, and 207.8±3.2 µm in kidney sections from 4-week-old C3H/HeN, BALB/cAnN, SJL/J, C57Bl/6N, DBA/2N, CD1 (CRI), 129S2/SvPas, CB6F1, FVB/N and NMRI (Han) mice, respectively (n=5 animals from each strain). The mean distance from the kidney surface of the most superficial glomeruli was significantly lower in the strains C3H/HeN Crl, BALB/cAnN, DBA/2NCrl, and C57Bl/6N when compared to a peer group consisting of all the other strains ($p<.0001$). In 10-week-old mice, the most superficial glomeruli were located deeper in the cortex when compared to 4-week-old animals, with BALB/cAnN and C57Bl/6N being the strains with the highest percentage of superficial glomeruli (25% percentile 116.7 and 121.9 µm, respectively). In summary, due to significantly more superficial glomeruli compared to other commonly used strains, BALB/cAnN and C57Bl/6N mice appear to be particularly suitable for the investigation of glomerular function using MPM.

Increased KGF Expression Promotes Fibroblast Activation in a Double Paracrine Manner Resulting in Cutaneous Fibrosis.

Johanna Canady, Stefanie Arndt, Sigrid Karrer, Anja K. Bosserhoff

Fibrotic disorders of the skin share the characteristic features of increased production and deposition of extracellular matrix components by activated fibroblasts. Their clinical course ranges from benign with localized cutaneous involvement to a systemic, life-threatening disease. The molecular cause for fibroblast activation remains unknown, yet epithelial-mesenchymal interactions draw mounting attention in the research field of fibrogenesis. We examined keratinocyte growth factor (KGF), a crucial molecule in fibroblast-keratinocyte cross talk, exemplarily in keloid and scleroderma, and found its expression to be increased in disease-derived fibroblasts and tissues compared with healthy controls. This overexpression induces fibroblast activation through a double paracrine mode of action. Upon KGF stimulation, the keratinocytes produced and secreted OSM (oncostatin M). Fibroblasts were in turn activated by OSM reacting with the increased expression of collagen type I-α1, fibroblast activation protein, and enhanced migration. The observed increase in collagen expression and fibroblast migration can be traced back to OSM-regulated STAT3 phosphorylation, leading to enhanced urokinase plasminogen activator expression. Hence, we propose a causative loop in the pathogenesis of fibrosing disorders of the skin mediated by the overexpression of KGF in mesenchymal cells.

The role of alpha Syntrophin in hepatic lipid metabolism

Kristina Eisinger, Sabrina Bauer, Markus Neumeier, Sandra Schmidhofer, Marvin E Adams, Stanley C Froehner, Christa Buechler

Objective: Alpha syntrophin (SNTA) is a multidomain adaptor protein which binds to other molecules due to its modular structure. SNTA interactive proteins like ATP-binding cassette transporter A1 (ABCA1) are expressed in liver but systemic and hepatic lipids have not been analysed in SNTA -/- mice to our knowledge so far.

Results: SNTA is expressed in the human and murine liver and in isolated hepatocytes. SNTB1 and SNTB2 members of the syntrophin protein family were not induced in liver of SNTA deficient mice excluding compensatory upregulation of these proteins. SNTA-/- mice kept for 24 weeks on a high fat diet showed no differences in bodyweight compared to wild type mice kept on same conditions. Systemic cholesterol and Apo B-100 of SNTA deficient mice kept on a high fat diet were similar to wild type mice while systemic triglycerides were increased. Liver triglycerides were not altered but hepatic cholesterol was significantly higher in the SNTA -/- mice. Hepatocytes isolated from the liver of SNTA-/- mice tended to have higher cholesterol level than cells from wild type mice. Activated form of sterol regulatory element binding protein 2 (SREBP2) and ABCA1 protein were, however, similarly abundant in the liver of SNTA-deficient and wild type mice. Stearoyl CoA desaturase (SCD1) catalyzes production of oleic acid from the saturated fatty acid stearic acid and is increased in the liver of SNTA-/- mice when kept on a standard chow and a high fat diet. In liver steatosis cellular triglycerides and cholesterol are increased but SNTA is not regulated.

Conclusion: These experiments suggest that SNTA and the pathways influenced by this adaptor protein in hepatocytes have a function in hepatic lipid homeostasis.

Molecular Function and Transport of MIA

Matthias Molnar, Jennifer Schmidt, Anja K. Bosserhoff

MIA (melanoma inhibitory activity) is a globular, secreted protein with a putative signal peptide. The mature MIA has a molecular weight of 11 kDa, and is highly expressed in melanoma cells but not in normal melanocytes. MIA is involved in the development, progression and metastasis of malignant melanoma and is currently used as a prognostic marker. Previous studies showed that MIA is secreted in a calcium-dependent manner at the trailing edge of melanoma cells, binds to matrix proteins and integrins and thereby promotes cell migration.

The main goal of my PhD project is to clarify the mechanisms by which MIA-transport and MIA-secretion are regulated. Therefore, we investigate different melanoma (Mel Im, Mel Ju, Mel Ei) as well as non-tumoric (HEK293, HeLa) cell lines.

To determine the influence of the signal peptide on MIA transport and secretion, we designed several MIA-signal peptide mutants and analyzed their effects on transport and secretion by Western Blot analysis and MIA-ELISA of cell culture supernatants. We also analyzed the different MIA mutants for localized secretion by immunofluorescence staining. The results show that the signal peptide is essential for MIA transport/secretion. Additionally, cleavage of the signal peptide seems to be important for MIA-secretion at the cell membrane.

Furthermore, we are aimed to isolate MIA-containing vesicles to elucidate the mechanism of MIA transport and secretion in more detail. To this end, we currently perform a variety of experiments including Size Exclusion Chromatography (SEC), different centrifugation assays and affinity chromatography. The aim of these experiments is to identify interacting transport- or adapter proteins of MIA and thereby to specify the assisting mechanisms of the vesicular traffic.

Taken together, this work will hopefully contribute to a deeper understanding of the molecular role and function of MIA.

Talk Part IV

InSilico analysis of medical and biological data using the *SISSi* software
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Deletion of TASK3 K^+ channels leads to hyperaldosteronism in adrenal glands of neonatal mice
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InSilico analysis of medical and biological data using the *SISSi* software

Torsten Schön, Martin Stetter, Erika Wichro, Natalie Lanner, Ruben Charchoghlyan, Karine Sargsyan, Ana Maria Tomé and Elmar W. Lang

Investigating interactions and relationships between clinical parameters with respect to the disease under study is a crucial task for retrieving knowledge about the disease. The huge amount of possibly important macroscopic and microscopic parameters and their higher order correlations leads to a combinatorial problem that can't be examined in common laboratory experiments.

The SustSol InSilico Simulator *SISSi* can extract a computer model out of parameters measured within a clinical study. SISSi examines the interactions and relationships underlying a dataset and represents the data as a Bayesian Network. Each parameter is represented as a node in the graph where nodes that depend on each other are connected by a directed edge. The values of a node are arranged in a limited set of categories, where for each category, a specific occurrence probability value is retrieved from the dataset. The nodes are further holding a conditional probability table with information of quantitative influence of their parents to the category probabilities.

Once a graphical network describing the underlying dataset has been learned, it is possible to simulate different scenarios by editing the probabilities of a node and observing the probability changes in the other nodes. For example, if a network describing nonalcoholic fatty liver disease (NAFLD) is containing a node for "NAFLD" state with two categories "Yes/No" has a parent node "Blood-Glucose-Concentration", one might increase the value of "Blood-Glucose-Concentration" and determine that the probability that node NAFLD is "Yes" has increased as well.

As an example, a novel NAFLD dataset containing patients and controls from the Medical University of Graz is analyzed using SISSi. After some cleaning steps, a graphical model is learned from the dataset and visualized in SISSi. The dataset is examined for unknown relations between dataset parameters and different scenarios are simulated.

Utilizing Patient Specific Induced Pluripotent Stem Cell Derivatives to Functionally Characterize HSPB7 Cardiovascular Risk Alleles

Maya Fuerstenau-Sharp, Martina Zimmermann, Nico Jentsch, Klaus Stark, Christian Hengstenberg

Dilated cardiomyopathy (DCM) is a common heart disease affecting the myocardium. The major characteristic of DCM is systolic dysfunction resulting in ventricular dilation as well as impaired function and eventually heart failure. While about 35% of patients have familial forms of the disease, it has been presumed that genetic factors also contribute to idiopathic cases of DCM. In order to diagnose DCM in its familial and idiopathic form to initiate treatment prior to its onset, the identification of risk alleles is crucial.

Through a genome-wide candidate gene study, *HSPB7* was recently identified as a candidate risk gene for idiopathic DCM. *HSPB7* encodes the small cardiovascular heat shock protein (cvHsp). Individuals homozygous for the *HSPB7* major allele (G) have a 30% increased risk to develop DCM while those homozygous for the *HSPB7* minor allele (A) are at a 40% decreased DCM risk. In addition, allele specific gene expression studies in heterozygous human cardiomyocytes have demonstrated that the major allele is expressed 10 fold less than the minor allele. These results suggest that HSPB7 might be a protective factor in cardiac muscle cells.

To functionally characterize *HSPB7*, we decided to utilize human induced pluripotent stem (iPS) cell derived cardiomyocytes to model DCM *in vitro*. This approach enables us to create a more relevant, disease-specific cellular model.

As a first step, we generated several iPS cell lines from an individual homozygous for the minor allele. Next, we transduced two of these iPS cell lines with a lentiviral construct carrying the ventricular specific promotor myosin light chain 2 (MLC2v) fused to an antibiotic resistance gene and a DOX inducible HSPB7 specific shRNA. This construct enables us to knock down *HSPB7* in pure ventricular cardiomyocytes. Thus far, we have already successfully differentiated several of these genetically engineered iPS cells lines into cardiomyocytes. We are currently in the process to optimize our selection protocol, with the goal to obtain pure ventricular cardiomyocytes. To assess *HSPB7* function in these cells, we will compare cardiomyocyte morphology, biochemistry and function pre- and post *HSPB7* knock down.

Connections between LMX1B-regulated proteins and the actin cytoskeleton

Natalya Lukajczyk, Tillmann Burghardt, Ralph Witzgall

Introduction: The transcription factor LMX1B belongs to the family of LIM-homeodomain proteins. Mutations in the *LMX1B* gene are associated with the nail-patella syndrome, a rare autosomal-dominant disorder affecting the development of the limbs, eyes, brain and kidneys. Microarray studies on glomeruli isolated from inducible podocyte-specific *Lmx1b* knock-out mice revealed a significant increase in the mRNA levels of several genes compared to control mice. Due to their time-course of induction and their common physiological function three promising LMX1B target genes were chosen for further investigation. While two of them, Abra (Actin-binding Rho activating protein) and Arl4c (ADP-ribosylation factor-like protein 4c), encode proteins that are associated with the actin cytoskeleton, the third candidate of the studies, Crct1 (Cysteine-rich C-terminal 1), is currently biologically unexplored, making it attractive as a research object.

Goal and methods: The main goal of the project is to identify the role of the putative LMX1B target genes in the development of the nail-patella syndrome. A crucial piece of information concerns the subcellular localization of the respective proteins and their interaction with the cytoskeleton in an immortalized human podocyte cell line. For this purpose cDNAs of Abra, Arl4c and Crct1 were fused with a HA-epitope tag and miniSOG, thus giving us the opportunity to visualize the proteins by immunocytochemistry as well as by electron microscopy.

Results: The studies revealed a co-localization of Abra and Arl4c with actin filaments in the tested cell line. MiniSOG fusion proteins confirmed these data and indicate a membrane-associated localization of Abra and Arl4c at the anterior pole of moving podocytes. Crct1 did not show specific specific localization within the cell. Interestingly, the level of F-actin decreased in the cells overexpressing Crct1.

Perspectives: In further studies we plan to localize the endogenous proteins in primary podocytes isolated from inducible podocyte-specific *Lmx1b* knock-out mice by use of antibodies raised against Arl4c, Abra and Crct1. Beside this, RNA interference studies on Abra, Arl4c and Crct1 are planned to obtain deeper insight how these proteins affect the actin cytoskeleton and the migratory properties of immortalized and primary podocytes.

The impact of mutated Fanconi-associated protein on the mitochondrial proteome

Aßmann N. , Klootwijk E. , Reichold M. , Reinders J. , Warth R. , Kleta R. , Oefner P.

The renal Fanconi syndrome is characterized by the failure of the proximal tubules in the kidney to reabsorb small molecules causing urinary loss of amino acids, glucose, electrolytes, phosphate, and low-molecular-weight proteins.

Recently, Kleta and coworkers at the University College London identified a novel form of autosomal dominant renal Fanconi syndrome (LOD-Score > 3) in an extended family by classical linkage analysis.

The EHHADH gene encodes for a peroxisomal protein, Enoyl-CoA hydratase/3-Hydroxyacyl-CoA dehydrogenase (EHHADH), which is involved in β-oxidation of fatty acids. The C-terminus contains the typical peroxisomal target sequence SKL. Upon mutation at the N-terminal end an exchange of a negatively charged amino acid, glutamic acid, by a positively charged amino acid, lysine, takes place, which originates a new mitochondrial targeting sequence. Due to this amino acid exchange, EHHADHmut is erroneously transported into the mitochondria as confirmed by immunohistochemistry.

The aim of the study is the elucidation of the molecular effects of the EHHADHmut-mislocalization to the mitochondria. The impact of the mislocated EHHADHmut is analysed using LLC-PK1 cells, which derive from the proximal tubulus. For this purpose, the cells were stably transfected with either EHHADHwt or EHHADHmut cDNA using the inducible Tet-On gene expression system. Cells start to express EHHADH within 24 hours after the addition of tetracycline and are grown for 7 days on glucose-free medium.

By means of differential proteome analyses of purified mitochondria and whole cell lysate the effect of the mutated EHHADH on the LLC-PK1 proteome is analysed employing two-dimensional gel electrophoresis and multi-dimensional liquid chromatography tandem mass spectrometry. Whole cell lysates were analysed by means of 2D-DIGE.

Ongoing characterization of the differentially regulated spots by nano-HPLC/QTOF-MS has led so far to the identification of proteins involved in remodelling of the cytoskeleton, e.g. actin, tubulin, and plastin, as well as proteins that are involved in energy metabolism, such as alpha-enolase and an electron-transfer-flavoprotein.

In the following, iTRAQ (isobaric tags for relative and absolute quantification) labelling in combination with mass spectrometry-based quantitative protemics will be employed to improve coverage of membrane proteome.

Deletion of TASK3 K^+ channels leads to hyperaldosteronism in adrenal glands of neonatal mice

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Background: Depolarisation of adrenal zona glomerulosa cells is a pivotal event for the secretion of aldosterone. Potassium channels like TASK1 and TASK3 are highly expressed in the adrenal cortex and determine the membrane voltage. TASK3$^{-/-}$ mice showed a severe age-dependent hyperaldosteronism. Therefore, the aim of this study was to identify the mechanisms underlying this age-dependent hyperaldosteronism.

Methods and Results: Neonatal TASK3$^{-/-}$ mice (ko) showed higher plasma aldosterone levels compared to wildtype (wt) animals correlating with an increased adrenal mRNA expression level of the aldosterone-synthase. These high aldosterone levels decreased within the first two weeks of life. Plasma concentrations of progesterone and corticosterone showed the same age-dependent dysregulation. These results pointed to a broader adrenal dysfunction in newborn ko mice. A chip-based analysis was performed to measure differential gene expression in adrenals of 1 and 12 day old mice. Interestingly, adrenal renin mRNA was strongly upregulated in newborn ko mice, but decreased to normal wt levels in 12 day old mice. The high renin gene expression was confirmed by realtime PCR and by renin-specific immunofluorescence. Measurements of renin activity in adrenal gland lysates revealed an increase of active renin in newborn ko mice. There was no difference in renal and plasma renin activity compared to wt animals.

Discussion and Perspective: These data suggest that transient activation of the local adrenal renin-angiotensin-system contributes to the hyperaldosteronism of neonatal TASK3$^{-/-}$ mice. Future studies are needed to investigate the signal pathways resulting in increased adrenal renin production and to identify the compensation mechanisms present in adult mice.

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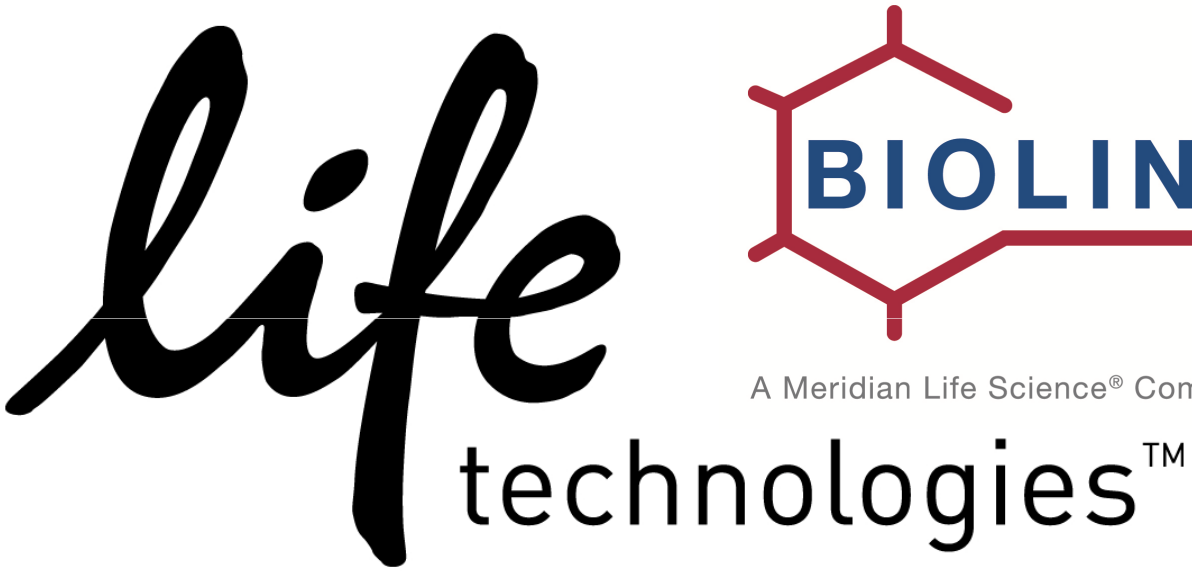

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