RIGeL Regensburg International Graduate School of Life Sciences

biomed regensburg international graduate school of life sciences

BIOMEDIGS BIOMEDICAL INTERNATIONAL GRADUATE SCHOOL

ABSTRACTS

BIOMEDICAL SYMPOSIUM

for Graduate Stundents

26th and 27th of September 2014

Universität Regensburg
FACULTY OF MEDICINE
FACULTY OF BIOLOGY AND PRECLINICAL MEDICINE

Herausgeber: Fakultät für Medizin
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http://www.uni-regensburg.de
Gestaltung: Konstanze Bedal

BIOMEDICAL SYMPOSIUM

for Graduate Students

26th and 27th of September, 2014

Tagungshaus Bayerischer Wald, Regen

BIOMEDIGS
BIOMEDICAL INTERNATIONAL GRADUATE SCHOOL

biomed
regensburg international graduate school of life sciences

RIGeL
Regensburg International
Graduate School of Life Sciences

Organisation:

BIOMEDIGS:
Konstanze Bedal
Verena Leidgens
Ute Mayer
David Stieglitz

RIGeL:
Nicole Bezhold
Katharina Gerl
Janina Staffel
Julia Stindl

FRIDAY, 26th of September 2014

8:30 DEPARTURE from Klinikum bus stop (main entrance)

10:00-10:30 CHECK-IN at Tagungsghaus "Bayerischer Wald" in Regen

10:30-10:45 WELCOME RECEPTION Janina Staffel

10:45-12:15 **TALK SESSION 1** Chair: David Stieglitz

1 **Isolation of infiltrative and stationary human brain tumor initiating cells in an in situ organotypic slice culture migration model**
Verena Leidgens

2 **Role of CYLD in early melanoma development**
Miriam De-Jel

3 **Molecular mechanisms of early cancer cell dissemination in breast cancer**
Hedayatollah Hosseini

4 **Molecular profiling of EpCAM-positive cells isolated from the bone marrow of breast cancer patients**
Nina Patwary

5 **The role of Exportin-5 in miRNA processing in malignant melanoma**
Corinna Ott

6 **Processing of the liver protein MIA2**
Mona Solanki

12:15-13:15 LUNCH

13:15-15:00 **TALK SESSION 2** Chair: Verena Leidgens

7 **p62 in CD40-mediated NFκB activation**
Kristina Seibold

8 **Genetic differences determining outcome of dextran sulfate sodium (DSS)-induced acute colitis in C57BL/6J and C57BL/6N mouse strains**
Nicole Bezold

9 **Computational Design of Epitope-Enriched HIV-1-Gag-Antigens for Induction of Broad T Cell Responses and their Experimental Assessment**
Johannes Meier

10 **Identification of C-Flip, Traf2, Nemo and Usp2 as interesting players for crosstalk in TNF and TRAIL signaling in HCC with the help of Boolean Nested Effects Models**
Martin-Franz-Xaver Pirkl

11 **Spike-in cell normalization accounts for global gene expression changes and controls RNA lysis**
Franziska Taruttis

12 **Improving supervised classifiers with unlabelled data using Autoencoders**
Anton Moll

15:00-16:00 MEET AN EXPERT
Prof. Dr. med. Hans-Joachim Anders

16:00-16:15 COFFEE BREAK

16:15-17:20 **POSTER SESSION 1**

1 **2-Hydroxyglutarate reduces IL-12 production of human dendritic cells**
Zugey Conejo

2 **Regulation of cellular dormancy in disseminated breast cancer cells**
Ana Grujovic

3 **New Insights into Muramyl Dipeptide (MDP) Effects on Human Dendritic Cells**
Carina Matos

4 **The role of downstream interaction partners in LKB1 signaling**
Olga Panichkina

5 **Cellular and molecular analysis of BMP6 during formation and progression of malignant melanoma**
David Stieglitz

17:20-18:10 **POSTER SESSION 2**

6 **Analysis of immune cell infiltration in GvHD target organs during acute GvHD**
Sakhila Ghimire

7 **Production of CMV immediate-early-1-protein as optimized antigen for T cell stimulation**
Richard Kiener

8 **Cell based epitope mapping for HIV-1 broadly neutralizing antibodies**
Veronika Schmid

9 **hBD2 activates human macrophages and synergizes in pro-inflammatory cytokine expression induced by TLR ligands**
Daniela Wanke

18:15 DINNER

20:00 GET TOGETHER

SATURDAY, 27th of September 2014

9:00- 9:30 Section Meeting BIOMEDIGS/RIGeL

9:30-11:00 **TALK SESSION 3** Chair: Julia Stindl

13 **The role of cell polarity regulator Par3/Bazooka in nephrocyte development**
Gudrun Mendl

14 **Ultrastructural analysis of Drosophila nephrocytes – Insights in podocyte development and disease**
Florian Hochapfel

15 **Natriuretic peptides have renoprotective effects after UNx by activating guanylyl cyclase A in podocytes**
Janina Staffel

16 **Chronic psychosocial stress induces inflammatory and anti-inflammatory responses**
Thi-Thu-Trang Nyguyen

17 **Anoctamins support calcium-dependent chloride secretion by facilitating calcium signaling in adult mouse intestine**
Podchanart Wanitschkool

18 **Volume regulation by anoctamins**
Lalida Sirianant

11:00-11:15 COFFEE BREAK

11:15-12:30 **POSTER SESSION 3**

10 **Characterization of the functional relevance of the local renin-angiotensin-system (RAS) in the renal collecting duct**
Anna Federlein

11 **Tamoxifen-inducible Cx40 cell specific Vhl deletion induces erythropoietin production in the kidney**
Katharina Gerl

12 **The role of cell type-specific type I collagen expression in renal fibrosis**
Simone Kutzi

13 **PATJ and its role in the Hpo-Pathway**
Thomas Roessler

14 **Physiology and pathophysiology of ATPases in aldosterone secretion**
Julia Stindl

15 **Mutation of the Fanconi-associated protein 2 (FAP2) as a cause of hereditary renal tubulopathy**
Julia Wieser

12:30-13:30 LUNCH

13:30-14:15 INDUSTRIAL TUTORIAL
PromoCell

14:15-15:30 **POSTER SESSION 4**

16 **The metabolite 5'-methylthioadenosine (MTA) signals through the adenosine receptor A2B in melanoma**
Katharina Limm

17 **Combining Computational Protein Design with Molecular Dynamics: A flexible approach to improve design accuracy**
Patrick Loeffler

18 **Interaction between osteoarthritic cartilage and chondrocytes/mesenchymal stem cells in vitro**
Ute Mayer

19 **The role of CMKLR1 and its ligands in non-alcoholic fatty liver disease**
Rebekka Pohl

20 **Estimating absolute abundances of bacteria in the intestinal microbiome**
Frank Stämmler

21 **The role of Syntrophin alpha and beta2 in Nonalcoholic Steatohepatitis (NASH)**
Lisa Voggenreiter

15:30-15:45 COFFEE BREAK

15:45-16:15 POSTER & TALK AWARD

ABSTRACTS

FRIDAY, 26th of September 2014

Isolation of infiltrative and stationary human brain tumor initiating cells in an in situ organotypic slice culture migration model

V. Leidgens[1], N. Patwary[2], E. Kerkhoff[3], M. J. Riemenschneider[4], U. Bogdahn[1], A. Vollmann-Zwerenz[1], C. A. Klein[2], P. Hau[1]

[1]Wilhelm Sander-NeuroOncology Unit and Department of Neurology, University of Regensburg, Regensburg, Germany, [2]Department of Experimental Medicine, University of Regensburg, Regensburg, Germany, [3]Department of Neurology, University of Regensburg, Regensburg, Germany, [4]Department of Neuropathology, Regensburg University Hospital, Regensburg, Germany.

Glioblastomas (GBM) are highly malignant brain tumors, hallmarked by infiltrating growth of tumor cells. According to recent publications, GBM pathogenesis may depend on Brain Tumor Initiating Cells (BTIC). In particular the subventricular zone and hippocampus are areas where BTICs may arise migrate to distinct areas and initiate the tumor bulk via differentiation into the glial linage. Therefore, it is relevant to investigate the transdifferentiation, migratory as well as invasive potential of BTICs through the identification of molecules and pathways controlling these mechanisms.

As a valuable tool an in situ model of organotypic brain slice cultures is utilized to visualize and analyse migration and invasion in a setting that simulates normal brain tissue conditions. This method allows the inoculation of tagged BTICs in the hippocampal region of fresh brain slice cultures from newborn rats (P8-14), and to monitor tumor cell invasion for up to three weeks. During this process the original cell population divides into different subpopulations: a stationary that proliferates at the implantation site, and an infiltrating one. Stationary and infiltrating cells differ in morphology and behaviour. In order to reveal why an initial cell population separates into subpopulations, a method was developed to microdissect single cells from the slices after an inoculation time of up to three weeks. cDNA libraries were generated for subsequent microarray analysis to compare the subgroups regarding gene expression profiles of genes associated with e.g. proliferation, invasion, cell-cell contacts, and stemness.

Besides the different phenotypic appearance of the subpopulations, the genetic pattern of cells from the subpopulations determined by microarray analysis should unveil genes driving tumor invasion. These newly identified targets will be further characterized and regulated to manipulate the invasive behaviour and tumorigenicity of BTICs.

Furthermore, the modulation of the transcription factor STAT3, which is a pivotal factor inducing transdifferentiation from epithelial to mesenchymal phenotype, potentially enabling BTICs to invade the surrounding tissue, will be investigated.

Role of CYLD in early melanoma development

M. de Jel, S. Kuphal, S. Schiffner and A. K. Bosserhoff

Molecular mechanisms of early cancer cell dissemination in breast cancer

Hedayatollah Hosseini[1], Lahiri Kanth Nanduri[1], Carolin Ehrl[1], Christian Werno[2], Mattias Maneck[3], Piero Musiani[4], and Christoph A. Klein[1, 2]

[1]Experimental Medicine and Therapy Research, [2]Fraunhofer Project Group, Personalized Tumor Therapy ITEM, [3]Institute of Functional Genomics Computational Diagnostics Group, University of Regensburg, Germany, [4]Aging Research Centre, Gabriele d'Annunzio University Foundation, Chieti 66013, Italy

Molecular profiling of EpCAM-positive cells isolated from the bone marrow of breast cancer patients

Nina Patwary

Introduction: Metastasis is the major cause of cancer-related death. The founder cells of metastases and their molecular characteristics are currently unknown. These cells evolve from disseminated cancer cells (DCCs) that spread before surgical removal of the primary tumor. DCCs are currently detected in the bone marrow (BM) of breast cancer (BC) patients by staining against epithelial markers, such as cytokeratins or the epithelial cell adhesion molecule (EpCAM). The surface marker EpCAM allows the isolation of viable cells and therefore isolation of cellular DNA and mRNA. However EpCAM+ cells can also be detected in the BM of healthy donors (HD) which hampers the identification of true metastasis founder cells among the EpCAM+ DCCs. Therefore the aim of this work was to identify and characterise true metastasis founder cells among the EpCAM+ cells isolated from the BM of BC patients.

Results: In total, we isolated 431 single EpCAM+ cells from cancer patients and 51 EpCAM+ cells from HD. After whole genome and whole transcriptome amplification 63% of cells had a good quality gDNA and 57% a good quality cDNA. Limited expression profiling of selected epithelial transcripts did not allow an assignment of true EpCAM+ DCCs among the EpCAM+ cells, but the usage of comparative genome hybridisation (CGH) does. Cells with balanced profile in mCGH were further analysed upon characteristics of primary tumour. 245 EpCAM+ cells from cancer patients were analysed for mutational status in PIK3CA gene and for expression of hormone receptor. Surprisingly, only 3% of cells expressed ESR1 transcripts. No cell expressed PGR. Only 2% of the cells harboured PIK3CA mutations. Comparing EpCAM+ cells from M0 BC patients and M1 BC patients regarding the mutational status and estrogen receptor expression both, PIK3CA mutations and estrogen receptor expression was observed more frequent in EpCAM+ cells from M1 BC patients. This difference between M0 and M1 stage DCCs was also seen for gene expression profiles. Here, cells from metastatic patients overexpressed differentiation and proliferation markers, which were not observed in M0-DCCs.

Discussion: Due to heterogeneity of EpCAM+ cells from cancer patients sufficient separation of true EpCAM+ DCCs from EpCAM+ cells from healthy donors by limited expression profiling is not possible, but mCGH does. The comparison of M0-stage DCCs to M1-stage DCCs indicates an ongoing genomic and trancriptomic adaptation.

The role of Exportin-5 in miRNA processing in malignant melanoma

Corinna Ott and Anja Bosserhoff

Introduction: MicroRNAs (miRNAs) are key players in the development of several kinds of diseases including cancer. It has already been shown that many miRNAs are deregulated in malignant melanoma, the most aggressive and fatal form of skin cancer, arising from melanocytes. In contrast to other cancers, the majority of miRNAs is upregulated in melanoma, leading to enhanced cell proliferation, migration and invasion. The reasons for this overexpression of miRNAs in melanoma are still not fully elucidated. Therefore, we tried to find out whether changes in the miRNA processing machinery could be responsible for the enhanced miRNA expression. Recently, it was reported that the expression of the miRNA processing enzymes DICER1 and DROSHA is reduced during melanoma progression.

Results: We investigated the potential role of the miRNA transporter Exportin-5 (XPO5) for the processing and maturation of miRNAs. In different melanoma cell lines, primary tumors and metastases of melanoma, XPO5 was found to be overexpressed compared to melanocytes. Thus, the effect of XPO5 downregulation and overexpression on different melanoma cell lines and melanocyte-like cell clones, respectively, was analysed.

After downregulation of XPO5, a slight decrease of mature miRNA level could be detected in melanoma cell lines. Interestingly, no substantial changes in cell proliferation or migratory potential could be found in two-dimensional assays after transfection. However, the capability of forming three-dimensional colonies out of one single adhesion-free cell was drastically influenced by XPO5 downregulation or overexpression, reflecting the connection between XPO5, 3D-proliferation and metastatic potential.

Discussion: These data show the importance of the miRNA transporter XPO5 on the regulation of miRNA expression and suggest a connection between XPO5 and metastasic potential in malignant melanoma.

Processing of the liver protein MIA2

Mona Solanki, Jacqueline Schlegel, Claus Hellerbrand and Anja Boßerhoff

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p62 in CD40-mediated NFκB activation

Kristina Seibold

Genetic differences determining outcome of dextran sulfate sodium (DSS)-induced acute colitis in C57BL/6J and C57BL/6N mouse strains

Nicole Bezold, Nadja Reul and André Gessner

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Computational Design of Epitope-Enriched HIV-1-Gag-Antigens for Induction of Broad T Cell Responses and their Experimental Assessment

Johannes Meier, Benedikt Asbach, Matthias Pfeifer, Thomas Schuster, Philip Rosenstock, Jens Wild, Josef Köstler and Ralf Wagner

Introduction:

The partially protective phenotype observed in HIV-1 infected long term non-progressors is often associated with certain HLA alleles thus indicating that CTL responses play a crucial role in combating virus replication. However, both the large variability of HIV, and the diversity of HLA alleles, impose a challenge on the elicitation of protective CTL responses by vaccination. To address this problem we designed Gag-based antigens which are optimized with regard to immunological breadth, while at the same time, the protein structure and the budding function were preserved. The optimized antigens were analysed regarding magnitude and breadth of their immune response.

Results:

A computational algorithm was employed to generate epitope-enriched Gag variants with patient-derived T cell epitopes (from the Los Alamos Molecular Immunology database) as input. To evaluate the performance of the algorithm, we generated a bivalent set of candidate Gag sequences (Opt1 and Opt2). In silico analysis shows, that these Gag variants resemble B-clade sequences due to a corresponding bias for B-clade-derived epitopes in the database. Regarding the epitope-coverage, both variants are superior to 95 % of all Gag-sequences from the LANL database, while the combination of both exhibits coverage of more than 99.9 %. Compared to the reference sequence HXB2, Opt1 contains 33, and Opt2 30 amino acid substitutions. Despite these substitutions both variants were, as intended, budding-competent. Preliminary immunological evaluation in a human ex vivo DC-model showed that the novel antigens were capable of stimulating T cell responses of the same magnitude as the wildtype protein. To demonstrate an increased immunological breadth we co-transfected HEK293T cells with soluble HLA class I molecules and the Gag variants. After affinity chromatography with the antibody W6/32, which only binds HLA class I molecules if they are in their natural complex with beta-2-microglobulin and an epitope, we isolated the epitopes and sequenced them via tandem mass spectrometry. Sequencing of epitopes would be a powerful tool for direct identification of HLA class I presented peptides without elaborate in vivo experiments.

Discussion:

Given the yet to be demonstrated increase in breadth, these designer Gag proteins qualify as next-generation antigens for induction of broader CTL responses. The algorithm has also the potential to optimize any antigen, provided enough CD8-T cell epitopes are known.

Identification of C-Flip, Traf2, Nemo and Usp2 as interesting players for crosstalk in TNF and TRAIL signaling in HCC with the help of Boolean Nested Effects Models

Martin Pirkl, Dominique Kranz, Grainne Kerr, Michael Boutros, Rainer Spang

Introduction: Understanding cell signaling pathways is key in battling cancer. Extensions and revisions of current pathway models can have immediate consequences on treatment strategies.

Saez-Rodriguez et al. (2009) propose the use of Boolean models and prior knowledge networks (PKN) to infer the pathway structure based on perturbation experiments and phosphorylation data. Nested effects models (NEM) by Markowetz et al. (2005, 2007) make inference about upstream-downstream positions of signaling genes from the subset relationship of downstream effects in global gene expression profiles. We propose a hybrid model to investigate the crosstalk of Tumor necrosis factor alpha (TNFα) and Tumor necrosis factor related apoptosis inducing ligand (TRAIL) in Hepatocellular Carcinoma Cell lines (HCC). Since TRAIL induces apoptosis in HCC but not normal cells, it has the potential to be used as a therapeutic. Active TNFα signaling on the other hand can desensitize cells for TRAIL induced apoptosis.

Results*: A pathway model with the highest fit to the gene expression data is resolved. It explains most of the data, considering noise. A look at the visualized best fitted data shows expression changes in the C-Flip and Nemo knockdowns which are incompatible to the Boolean modeling approach and therefore are accounted for in the learning algorithm as mismatches. In the Usp2, Traf2 and again C-Flip knockdowns significant expression changes happen during double stimulation exclusively, which can be modeled with a change in the PKN.

Discussion*: Our modeling approach confirms most of the literature. The qualitative Boolean approach, while very robust, naturally has problems with quantitative regulation (C-Flip, Nemo). A look at the fitted data and its residuals still helps to identify such regulation. Our model also hints at the possibility that C-Flip, Traf2, Usp2 and Nemo are involved in a crosstalk between TNFα and TRAIL.

**alternatively: research plan*

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Spike-in cell normalization accounts for global gene expression changes and controls RNA lysis

Franziska Taruttis, Rainer Spang, Julia Engelmann

Motivation:

Current gene expression protocols assume a constant amount of total RNA.

But recently it was found that global gene expression changes are possible, for example caused by transcriptional amplifiers, which amplify the expression of the currently expressed genes depending on the epigenetic background.

This might be a result of a mutation activating a promotor.

Due to increased gene expression of the majority of transcribed genes, the amount of RNA of cells with transcriptional amplification will be higher than in cell without transcriptional amplification.

Hence we cannot expect that the total amount of RNA remains constant across samples.

This behavior causes a violation of the main assumptions in present gene expression normalization methods.

Current normalization methods assume that only a small amount of genes changes expression, while most of the genes stay constant.

Ignoring these violations might lead to misinterpretation of the gene expression data if there are global gene expression changes.

In addition to that the RNA extraction is not well reproducible, which means that the amount of RNA extracted from replicated experiments can have a large variance.

We believe that there is no possibility for correct adjustment without controlling for the RNA lysis.

Results:

To account for these effects and to normalize data with different amounts of RNA, we add an external standard to our samples.

Since we want to control for RNA lysis, too, we spike-in foreign cells before the lysis protocol starts to control all experimental steps.

We showed that global gene expression changes are detectable using our spike-in cell normalization.

In dilution experiments we found that our spike-in normalization improves over normalization methods which do not use an external standard.

Improving supervised classifiers with unlabelled data using Autoencoders

Anton G. Moll and Claudio Lottaz

Introduction: One aspect of Personalized Medicine is to select out of many drugs only those that promise efficacy in the patient. The patients can differ in their genetic background, (sub-)type of disease, or in the environment that they were exposed to, giving rise to a difference in gene expression, which can be measured using microarrays. In addition, the response of a particular patient to a certain drug can be measured experimentally, giving the patients either a „sensitive" or „resistant" class label.

A simple approach to predicting the class label of a new patient is to train on the expression data of patients with known label. This approach has the shortcoming that usually there are not many labelled patients.

Results: We therefore propose to incorporate unlabelled data. Recently, it has been shown in image classification that pre-training an artificial neural network with unlabelled data improves discrimination performance in a supervised learning task significantly. We want to transfer this to drug efficacy prediction using several hundred human expression data sets for pre-training.

An autoencoder is a type of neural network. It can be used to obtain a compressed representation of its input while minimizing the loss of its reconstruction. We consider learning an autoencoder to find a network structure which compresses representations. This does not need labels. We then feed the data sets for which a label is available through the autoencoder, obtaining a compressed representation, and learn a classifier on it and the class label. This classifier can then be used to obtain predictions for new patients.

Learning an autoencoder is too lengthy when done on a single computer. The publicly available software ‚deepnet' can perform computations in parallel on shared-memory architectures (PC graphics cards), but would take too long on distributed-memory architectures (computing clusters). We therefore propose how to extend the matrix library of ‚deepnet' so that it can perform computation efficiently on high-performance computing clusters as well.

Discussion: Compressing the input space using an autoencoder, and then performing supervised learning on the compressed representation might increase the accuracy of drug efficacy prediction.

The extension of the matrix library has the added benefit that each of the computations which are performed in parallel on a cluster can be performed in parallel on a graphics card in each of the cluster's nodes.

2-Hydroxyglutarate reduces IL-12 production of human dendritic cells

Zugey Elizabeth Cárdenas Conejo, Marina Kreutz, Eva Gottfried

1

Introduction: Metabolic alterations have been described for many cancers. Monoallelic point mutations in isocitrate dehydrogenase (IDH) 1 and 2 are frequently found in neoplasias including acute myeloid leukemia and glioma, with different impact on prognosis and overall survival. IDH mutations are able to stimulate proliferation and suppress hematopoietic differentiation in cell culture leukemia models. IDH mutant enzymes lead to the production of R-2-hydroxyglutarate (2-HG), which has also been shown to recapitulate the effects of mutated IDH. However, effects of 2-HG on differentiation of non-malignant hematopoietic cells have not been described so far.

Results: Here, we analyzed the effect of 2-HG on different human myeloid cells, such as freshly isolated monocytes, monocyte-derived macrophages and monocyte-derived dendritic cells. Cells were stimulated with LPS in the absence or presence of R-2-HG or its enantiomer S-2-HG for 24 hours. TNF and IL-6 production were not altered in monocytes, macrophages and dendritic cells. In dendritic cells, the expression of maturation associated surface molecules like HLA-DR, CD80 and CD86 was also not affected by 2-HG treatment. However, we found that R-2-HG and S-2-HG decreased the production of IL-12(p70) by LPS activated dendritic cells at a concentration of 10 mM 2-HG, a physiological relevant concentration which has been detected in glioma and breast carcinoma. IL-12(p70) is composed of two subunits, p35 and p40. We analyzed both subunits by flow cytometry and found that p40 was down regulated on protein level by R-2-HG whereas p35 was not affected. In contrast, R-2-HG and S-2-HG suppressed both subunits, encoded by the genes IL-12A and IL-12B, on mRNA level. To elucidate underlying mechanisms responsible for the transcriptional repression by 2-HG, we studied signaling molecules involved in LPS signal transduction. Preliminary data indicate that the degradation of I-2B as well as the phosphorylation of AKT are not affected by R-2-HG and S-2-HG. LPS stimulation of dendritic cells has been shown to induce HIF-1a which is also discussed to be modulated by 2-HG. Our results support this hypothesis as R-2-HG and S-2-HG destabilized HIF-1a in dendritic cells.

Discussion: In summary, both 2-HG enantiomers significantly affect the production of IL-12(p70) in dendritic cells, which might be important for immunosuppression in the tumor environment.

Regulation of cellular dormancy in disseminated breast cancer cells

Ana Grujovic

Despite extensive research and development of novel therapies, cure of solid cancers is still achieved only by surgery - if at all. Supporting adjuvant therapies such as chemotherapy and immunotherapy have limited success and many patients die from metastases that may arise long after eradication of their primary tumour. This latency period between detection of primary tumours and first metastases led to the idea that in these patients the residual disease may have been in a dormant state. The term dormancy implies that the disease can re-awake and progress to a life-threatening condition. DCCs which can be detected in and isolated from bone marrow of patients with epithelial cancers are mostly negative for markers of proliferation, suggesting that they are in a state of cellular quiescence and may stay dormant for more than 10 years. Since detection of DCCs correlates with the subsequent development of distant metastasis, they are thought to comprise the precursor cells of metastasis. The analysis of single disseminated breast cancer cells revealed that systemic cancer spread starts extremely early in the genomic progression of cancer. Metastatic dissemination often takes place before the onset of chromosomal instability. These data were integrated into a novel model of systemic cancer progression. These findings also added to a better understanding of cancer dormancy.

The aim of our study is to identify the mechanisms that regulate cellular dormancy in bone marrow of breast cancer patients. We want to identify epigenetic and transcriptional factors that initiate and maintain dormancy, study their activation by environmental signals and finally address how cells exit the state of quiescence.

Hypotheses

1) Dormancy is a state that is governed by cell-intrinsic and environmental-extrinsic factors.

2) Cellular states of dormancy and proliferation can be identified in single DCC by gene expression profiling.

Aims

1) Establishing in vitro and in vivo models of cellular dormancy

2) Generating gene signatures for functional states of dormancy and proliferation

3) Identifying the dormancy model that fits best to DCCs isolated from bone marrow of the patients

4) Functional analysis of key players in dormancy signatures

New Insights into Muramyl Dipeptide (MDP) Effects on Human Dendritic Cells

Carina S. Matos, Kathrin Renner, Katrin Peter, Ernst Holler, Marina Kreutz

3

The role of downstream interaction partners in LKB1 signaling

Olga Panichkina, Michael Krahn

Introduction: LKB1 tumor suppressor was first determined in 1998 in patients with Peutz-Jeghers syndrome (PJS), an inherited, autosomal dominant disorder, characterized by gastrointestinal polyps and enhanced cancers risk of different organs. The LKB1 gene encodes a serine-threonine kinase (STK11), which phosphorylates adenosine monophosphate–activated kinase (AMPK) to suppress cell growth and proliferation as well as enhanced fatty acid oxidation and glucose uptake. On the other hand, the ability of LKB1 to inhibit mechanistic target of rapamycin (mTOR), a central player in cell growth and proliferation, results in cell cycle arrest. Moreover, LKB1 has been shown to regulate p21 levels and p53-dependent apoptosis pathways, inhibit transforming growth factor-beta (TGF-beta)/bone morphogenic protein (BMP) signaling and downregulate the Wnt signaling.

Beside its implications in cell cycle and proliferation control, LKB1 is known to control cell polarity and microtubule stability in epithelial, neuronal and other tissues both in Drosophila and vertebrates. Mutations in LKB1 affect asymmetric division of Drosophila larval neuroblasts and thus the central nervous system functionality.

Results: Despite some LKB1 downstream targets over last several years were investigated in detail, the complete regulation of LKB1-mediated pathways still remains perplexing. Thus, our study is focused on examining possible interaction partners/substrates of LKB1. Using a mass-spectrometry-based approach, we identified several new interaction partners of LKB1, which have been verified to be phosphorylated by LKB1 in vitro. We are currently mapping the phosphorylation sites and investigating possible impacts of non-phosphorylated and constitutively phosphorylated versions of the LKB1 substrates on the proteins localization and function.

Cellular and molecular analysis of BMP6 during formation and progression of malignant melanoma

David Stieglitz

5

Analysis of immune cell infiltration in GvHD target organs during acute GvHD

Sakhila Ghimire and Ernst Holler

Introduction: Hematopoietic stem cell transplantation remains an ultimate therapy to treat high risk hematological malignant disorder but its efficacy is limited by the occurence of graft versus host disease (GvHD). GvHD occurs when donor CD4+ T cells subsets get activated in response to host antigen presenting cells, recognize patient tissue as foreign and attack several organs like skin, liver, and most intensively, gut. Numerous studies in animal models and in human PBMCs have reported significant depletion of cells expressing the regulatory gene, FOXP3, and upregulation of inflammatory cells expressing TH1 or TH17 cytokines. Although urgently needed, the investigation of immune cell infiltrates in human tissues like regulatory T cells (FoxP3+ve) and inflammatory T cells like Th17 (CD4+ve, IL-17+ve) is highly constricted due to the lack and scarcity of patient materials. Therefore, the aim of this study is to analyze the cellular nature and source of regulatory proteins like inodlamine-2,3-dioxygenase (IDO) and FoxP3, and of inflammatory protein, IL-17, in a large cohort of patient biopsies obtained prior to and during acute GI GvHD. Futhermore, we also aim to analyse several relevant micro RNAs in gut biopsies to unravel the possible micro RNA signature involved in pathophysiology of aGvHD.

Results: Single antibody immunohistochemistry revealed stagewise upregulation of FoxP3- and downregulation of IL-17 positive cells in 145 transplanted patients which is in contrast to existing reports and introduces new hypotheses. Interestingly, a positive correlation between two regulatory parameters, IDO and FoxP3, was observed. The exact cellular source of FoxP3 positive cells and IL-17 positive cells was analyzed in 20 biopsies (a pilot study) exploiting double immunofluorescence. Existence of CD4 negative FoxP3 cells and CD4 negative IL-17 cells became evident. Transcription factor for IL-17 producing cells, RORc, was analysed at mRNA level on 169 biopsies where significant downregulation of RORc was observed during GI GvHD which is in parallel to depletion in IL-17. Nine micro RNAs were analysed in 31 gut biopsies out of which miR-155 was significantly upregulated in severe acute GvHD when compared with GvHD free patients after transplantation.

Discussion: For the first time, in the field of aGvHD, we have shown that regulatory phenomena involving FoxP3 increases and inflammatory phenonema involving IL17 decline along with RORc decline during acute GvHD, suggesting that immune infiltrates reflect the attempt to restore immune hemostasis. The association of FoxP3 and IDO positive cells suggests a regulatory feedback mechanisms in response to TH1 cell activation. Existence of CD4 negative FoxP3 cells and CD4 negative IL-17 cells suggest existence of further regulatory cells such as IL17 positive innate lymphoid cells which may be needed for intestinal homeostasis and could be modulated for treatment of GvHD in the future. Finally, upregulaton of miR-155 in pilot study suggested its importance in acute GI GvHD and a need to analyse miR-155 in a larger cohort. More research is definitely required to understand the complex intestinal immunoregulation in GvHD.

Production of CMV immediate-early-1-protein as optimized antigen for T cell stimulation

Richard Kiener, Benedikt Asbach and Ralf Wagner

T Lymphocytes are critically involved in long-term control of Cytomegalovirus infection. CMV reactivation due to immunosuppression is the main infectious complication in transplant recipients and is associated with T cell depletion. Due to the crucial role of T Lymphocytes in maintaining control over CMV, the degree of virus-specific T cell responses is most probably an important biomarker to monitor transplant recipients for risk of recrudescence. Enzyme-linked immunospot assays allow fast identification of CMV-reactive T cells in a sensitive as well as reproducible manner and are therefore suitable for widespread diagnostic use. As the crucial component for the fidelity of the assay is the stimulating antigen we wanted to establish an optimized procedure for production of highest-quality IE-1 protein, especially addressing the problem of LPS contamination.

Here, eukaroytic HEK293F cells were transiently transfected with pcDNA3.1-IE-1-Tag (C-terminal fusion of IE-1 with a tandem affinity tag) using polyethylenimine. IE-1 was purified from crude cell lysates by two subsequent affinity purifications. Purity was assessed by both Coomassie and silver staining of SDS-gels and protein yield as well as endotoxin levels were determined via Bradford and Limulus assay, respectively. PBMC or whole blood cultures were stimulated with IE-1 purified from mammalian cells or E. coli and IFN-γ positive T cells were identified via ELISPOT or intracellular cytokine staining followed by flow cytometry analysis.

The IE-1 preparation from 293F cells yielded approximately 0.65 mg/l culture volume. Silver staining of an SDS gel confirmed excellent protein purity. The LPS level was below the limit of detection in a Limulus assay (<0,5 EU/ml). Flow cytometry and ELISPOT assays both showed that the purified protein allows sensitive detection of IE-1 specific T cells from blood samples of CMV positive donors. At the same time the background signals, tested with blood from CMV seronegative donors, were markedly reduced.

In conclusion, the IE-1 protein purified from 293F cells is superior regarding specificity due to lower background signals. The purified protein showed similar stimulatory capacity for T cells as compared to IE-1 purified from E. coli. We also conclude that production of proteins meeting quality requirements for diagnostic use in mammalian cells is a viable alternative to bacterial systems, especially when endotoxin-depletion during purification is challenging.

Cell based epitope mapping for HIV-1 broadly neutralizing antibodies

Veronika Schmid, Alexander Kliche, Krystina Beer and Ralf Wagner

Introduction Due to the lack of a proof-reading activity in the HIV-1 reverse transcriptase and the human RNA polymerase, HIV-1 has an immensely high mutation rate. As a result of its high variability, most antibody responses are only strain-specific and can be bypassed quickly by escape mutations. However, after several years of infection, 10 – 20 % of all patients develop so called broadly neutralizing antibodies (bNAbs). Those bNAbs target the HIV-1 surface protein Env and can neutralize a broad variety of different HIV-1 isolates. Although bNAbs cannot prevent disease progression in those patients, their passive application has proved to protect macaques from SIV infection. However, none of the currently available Env immunogens have achieved an induction of bNAbs in immunization trials so far. Therefore, we aim to develop novel Env immunogens with enhanced affinity towards bNAbs, based on the hypothesis that those would be better suitable for the induction of bNAbs.

Results Using an Env Alanine library in a FACS based, high throughput screening platform on the bNAbs PG9 and VRC01, amino acid positions important for Env–bNAb binding were identified. A variety of statistically significant loss of binding mutations was identified, as well as one statistically significant gain of binding mutation for each antibody, enhancing the binding by a factor of 2.4 for PG9 and 1.8 for VRC01, respectively. To achieve higher enhancement rates, those gain of binding positions as well as selected loss of binding positions on the surface of Env are currently permutated to detect potential amino acids at those positions that lead to an even better binding. In addition, selected mutations on the surface of Env are currently investigated functionally.

Discussion Because of its lack of electric charge and chemical reactivity, alanine libraries are a useful tool to detect important amino acids for binding interactions in the form of loss of binding mutations. The permutation of those loss of binding positions might expose favorable mutations for enhanced binding. As the gain of binding mutation for the bNAb VRC01 is located at the edge of the epitope, the mutation into the small alanine might result in better epitope accessibility for VRC01. The gain of binding mutation for PG9 is located between the inner and outer domain of Env and cannot be in direct contact to PG9. However, it might lead to a favorable structural rearrangement of the bNAb epitope.

hBD2 activates human macrophages and synergizes in pro-inflammatory cytokine expression induced by TLR ligands

Daniela Wanke and Thomas Hehlgans

Introduction: Members of the beta-defensin super-family are small, cationic, antimicrobial polypeptides characterized by their tertiary structure consisting of a N-terminal α helix, followed by a β-sheet, and six highly conserved intramolecular cysteine residues. Beta-defensins are mainly expressed by cells of epithelial origin but also by immune cells and contribute to the innate immune response against Gram-positive and Gram-negative bacteria, fungi and some viruses. Beside their antimicrobial effect certain members of the beta-defensin super-family are able to modulate also the adaptive immune response. Previous studies showed that human beta-defensin 2 (hBD2) chemoattracts immature dendritic cells via CCR6 and interacts with CCR2, a chemokine receptor expressed on monocytes, macrophages, and neutrophils.

However hBD2-induced signaling pathways are poorly understood and possible mechanisms of synergy between beta-defensins and TLR ligands in macrophage activation have not yet been defined.

Results: Peripheral blood monocytes were separated by leukapheresis of healthy donors followed by density gradient centrifugation and subsequent counter current centrifugal elutriation. Macrophages were generated from isolated monocytes according to standard protocols for 7 days. The human macrophages were pre-stimulated with recombinant, biological active hBD2 and re-stimulated with the TLR ligands LPS and poly I:C. This treatment results in a synergistic, enhanced expression of the pro-inflammatory cytokines TNFα, IL6 and IL8. Furthermore, this synergistic effect was abrogated using Pertussis toxin, an inhibitor of Gi protein coupled receptors, indicating that the synergistic effect seems to be mediated by Gi protein coupled receptor signaling.

Discussion: Several studies showed that beta-defensins induce chemotaxis via the chemokine receptors CCR6 and CCR2. Our new results indicate that the synergistic, enhanced expression of the pro-inflammatory cytokines after hBD2 pre-stimulation and subsequent re-stimulation with TLR ligands is also dependent on Gi protein coupled receptor signaling. Our findings make it plausible that hBD2, which is higly induced after bacterial challenge, chemoattracts macrophages to the side of inflammation and furthermore supports the inflammatory reaction by enhancing pro-inflammatory cytokine expression induced by TLR lignads.

ABSTRACTS

SATURDAY, 27th of September 2014

The role of cell polarity regulator Par3/Bazooka in nephrocyte development

Gudrun Mendl and Michael Krahn

Introduction: The nephrocyte is a highly specialized cell type in Drosophila melanogaster which is responsible for the filtration of the haemolymph, thus removing harmful substances by endocytosis and life-long storage inside the cell. Resembling the mammalian podocyte in structure and function, the nephrocyte forms a size- and charge-selective filtration slit diaphragm and develops a network of channels in the cell periphery. A set of homologous proteins involved in forming the characteristic structures of these cells predestine the fly model for basic kidney research.

In a previous study, the cell polarity regulator Par3 has been shown to interact with Nephrin/Neph1 to cluster these proteins at the slit diaphragm. We have shown that RNAi knock-down of the Drosophila homologue of Par3 (Bazooka, Baz) has a strong effect on the correct development of the larval nephrocyte, manifesting in reduced numbers of filtration slit diaphragms and channel networks. In addition, first experiments indicate an impaired interaction of a phosphorylation-mutant Bazooka with the Nephrin-complex.

Results: In a newly established functional assay nephrocyte functionality can be quantified using confocal microscopy. Transgenic fly larvae secrete GFP into their haemolymph, which is taken up by the nephrocytes. GFP intensity levels in the nephrocytes differ depending on their ability of endocytosis and storage. A simultaneous RNAi knock-down of Baz and Baz-associated proteins resulted in a decreased level of cell fluorescence, indicating an impaired functionality. Furthermore, Immunostainings of larval nephrocytes showed an altered localization of Kirre/Neph1 when phosphorylation-mutant Bazooka is overexpressed.

Discussion: The decrease in nephrocyte functionality and ultrastructural integrity upon the knock-down/mutation of Baz and associated proteins indicate their substantial contribution to proper nephrocyte development. In addition, the phosphorylation status of Bazooka affects the stability and integrity of the slit diaphragms and filtration capability of nephrocytes. We therefore speculate that the establishment of the filtration barrier is dependent on the interaction of Par3/Bazooka and the Nephrin-complex.

Ultrastructural analysis of Drosophila nephrocytes – Insights in podocyte development and disease

F.Hochapfel, M.Krahn

Introduction

Drosophila nephrocytes are podocyte-like mesoderm-derived cells with filtration slit diaphragms and a complex network of labyrinthine channels in the cell periphery. They are located inside the fly body cavity performing haemolymph filtration, thereby taking up toxins and wastes into the channel system in a size- and charge-selective manner, followed by endocytosis, life-long storage and thus inactivation. Drosophila homologs of NPHS1 and NEPH1 are required for slit diaphragm formation and function. In the course of this work, expression levels of homologs to mammalian genes related to podocyte function or disease as well as cell polarity are specifically decreased or elevated in Drosophila nephrocytes using the UAS/GAL4-System. Shortly before puparation, larval garland nephrocytes are obtained and embedded in epoxy resin, followed by ultra-thin sectioning and transmission electron microscopy. Afterwards, the extent and complexity of the channel network and is quantified.

Results

A null-mutation (Δ1) of the myosin regulating factor and CPD downstream target PATJ severely impairs the development of both filtration slits and channel networks. The overall number of diaphragms is reduced and the distinct transition between the channel layer and the intracellular area is lost. Analyses of podocyte-related genes showed strong differences in channel complexity and depth.

Discussion

Given the numerous similarities with podocytes, nephrocytes are well-suited to serve as an efficient model for fundamental podocyte research. Their morphological complexity can be used as a sensitive screen for genes, relevant for podocyte development and function. As an epithelial cell, they can also be employed to study cell polarity dynamics.

Natriuretic peptides have renoprotective effects after UNx by activating guanylyl cyclase A in podocytes

Janina Staffel, Daniela Valletta, and Frank Schweda

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Chronic psychosocial stress induces inflammatory and anti-inflammatory responses

Trang Thi Thu Nguyen, Dominic Schmidt, and Daniela N. Männel

Introduction: Chronic stress leads to immune suppression lowering the production of antigen-specific antibodies in immunized individuals. During CSC (chronic subordinate colony stress, a clinically relevant model for chronic psychosocial stress), mice respond with systemic inflammation as evidenced by spontaneous colitis, stimulated lymphocytes with enhanced capacity for cytokine production, enhanced numbers of immature myeloid cells, and a higher number of germinal centers in the spleen. In this study, we tested how CSC influences the immune status and antibody production in vaccinated mice.

Results: Sera of mice after 19 days of CSC were tested for immunglobulin levels by ELISA and auto-reactive IgG on Hep-2 cells. Directly after CSC mice had higher levels of total IgG with a characteristic pattern of auto-reactive IgG Mice were immunized with ovalbumin (OVA, 50µg, into the hind foot pad) and blood, spleens, and lymph nodes of immunized stressed and non-stressed animals were analyzed ten days later. Immunized stressed animals produced more OVA-specific IgG and the activated state of the immune system was maintained as seen by an increased proportion of myeloid cells and activated T cells and a higher number of germinal centers in the spleen. Numbers of germinal centers in draining lymph nodes from the immunized and the contralateral side were elevated in stressed immunized mice.

Discussion: Chronic subordinate colony stress (CSC) leads to auto-antibody production, B cell activation as seen by isotype switching and higher antigen-specific IgG production after immunization while at the same time anti-inflammatory cell types are present.

Anoctamins support calcium-dependent chloride secretion by facilitating calcium signaling in adult mouse intestine

Rainer Schreiber, Diana Faria, Boris V. Skryabin, Podchanart Wanitchakool, Jason R. Rock and Karl Kunzelmann

Introduction: Intestinal epithelial electrolyte secretion is activated by increase in intracellular cAMP or Ca2+ and opening of apical Cl– channels. In infants and young animals, but not in adults, Ca2+-activated chloride channels may cause secretory diarrhea during rotavirus infection. While detailed knowledge exists concerning the contribution of cAMP-activated cystic fibrosis transmembrane conductance regulator (CFTR) channels, analysis of the role of Ca2+-dependent Cl– channels became possible through identification of the anoctamin (TMEM16) family of proteins. We demonstrate expression of several anoctamin paralogues in mouse small and large intestines.

Results: Using intestinal-specific mouse knockout models for anoctamin 1 (Ano1) and anoctamin 10 (Ano10) and a conventional knockout model for anoctamin 6 (Ano6), we demonstrate the role of anoctamins for Ca2+-dependent Cl– secretion induced by the muscarinic agonist carbachol (CCH). Ano1 is preferentially expressed in the ileum and large intestine, where it supports Ca2+-activated Cl– secretion. In contrast, Ano10 is essential for Ca2+-dependent Cl– secretion in jejunum, where expression of Ano1 was not detected. Although broadly expressed, Ano6 has no role in intestinal cholinergic Cl– secretion. Ano1 is located in a basolateral compartment/membrane rather than in the apical membrane, where it supports CCH-induced Ca2+ increase, while the essential and possibly only apical Cl– channel is CFTR.

Discussion: These results define a new role of Ano1 for intestinal Ca2+-dependent Cl– secretion and demonstrate for the first time a contribution of Ano10 to intestinal transport.

Volume regulation by anoctamins

Lalida Sirianant, Jiraporn Ousingsawat, Podchanart Wanitchakool, Rainer Schreiber, Karl Kunzelmann

Introduction: Volume regulation is an intrinsic property of any living cell. An increase in intracellular osmolytes or decrease in extracellular osmolarity leads to cell swelling, which is counteracted immediately by activation of K+ and Cl- currents, releasing KCl to the extracellular space. Anoctamin 1 (Ano1; TMEM16A) has recently been identified as the important Ca2+ activated Cl- channel (CaCC) expressed in a wide range of tissues and was found together with other anoctamins to support cellular volume regulation and regulatory volume decrease (RVD). However, the biophysical properties of Ano1 and its Ca2+ sensitivity made it an unlikely candidate for the classical volume regulated anion channel VRAC.

Results*: We present evidence that full activation of RVD and VRAC in HEK293 cells and lymphocytes requires the presence of physiological extracellular Ca2+ concentrations. Anoctamins are broadly expressed which makes them good candidates for VRAC. We analyzed mRNA expression of the ten anoctamins in a large number of tissues and cell lines and found that almost all human and murine cell types express Ano6, 8, 9, and 10. Whole cell currents induced by hypotonic cell swelling were measured using a patch pipette filling solution with a cytosolic-like ion composition, and physiological extracellular Ca2+ concentrations, allowing direct comparison of ion current and volume measurements. When overexpressed in HEK293 cells Ano6, 9, and 10 but not Ano8 enhanced RVD and VRAC as well as apoptotic whole cell currents induced by staurosporine.

Discussion*: The data suggest broadly expressed Ano6, 9, and 10 as possible candidates for swelling activated ion channels.

Characterization of the functional relevance of the local renin-angiotensin-system (RAS) in the renal collecting duct

Anna Federlein and Frank Schweda

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Tamoxifen-inducible Cx40 cell specific Vhl deletion induces erythropoietin production in the kidney

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Introduction: Based on previous evidence that conditional deletion of von Hippel-Lindau protein (pVHL) in renin-expressing juxtaglomerular cells of the kidney suppresses renin expression and induces erythropoietin expression, we wondered if this striking endocrine shift is developmentally programmed or if it can also be induced in the normal adult kidney.

Methods: To address this question we used a tamoxifen-inducible Cre deleter under the control of the connexin 40 gene promoter, as connexin 40 is strongly expessed by juxtaglomerular cells. Thus we generated $Vhl^{flox/flox}$ x $Cx40^{tam\text{-}Cre}$ mice, to induce Cx40 cell specific pVHL deletion at a particular time by tamoxifen supply. $Vhl^{+/+}$ x $Cx40^{tam\text{-}Cre}$ litters served as controls. Both genotypes were fed a special chow containing tamoxifen at the age of four weeks.

Results: Determination of hematocrit (hct) values of $Vhl^{flox/flox}$ x $Cx40^{tam\text{-}Cre}$ mice showed pronounced polycythemia (hct 65.9 ± 1.3%), that was caused by elevated red blood cell counts. Control litters had normal hct values (hct 47.0 ± 0.6%). The stimulated erythropoiesis was paralleled by elevated plasma EPO concentrations (2146.8 ± 443.8pg/ml). To search for the origin of EPO, we determined EPO mRNA expression in several organs, since Cx40 is apart from the kidneys also expressed to a significant extent in the arterial endothelium, in lung or heart. Amongst these organs we only found elevated EPO-mRNA levels in the kidneys and there especially in preparations of isolated glomeruli. In parallel renin mRNA levels, plasma renin concentrations as well as juxtaglomerular renin immunoreactivity were significantly reduced in these $Vhl^{flox/flox}$ x $Cx40^{tam\text{-}Cre}$ mice.

Discussion: Taken together our data might suggest that deletion of pVHL in juxtaglomerular cells of adult kidneys induces EPO and suppresses renin expression. Since mesangial cells also express Cx40 abundantly we have to clarify, if in tamoxifen treated $Vhl^{flox/flox}$ x $Cx40^{tam\text{-}Cre}$ mice these cells are able to express EPO as well.

The role of cell type-specific type I collagen expression in renal fibrosis

Simone Kutzi and Matthias Mack

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PATJ and its role in the Hpo-Pathway

Thomas Rössler

Introduction: The Hpo-Pathway is a conserved pathway regulating tissue and organ growth in many tissues by controlling proliferation and apoptosis. The Pathway consists of a core kinase cascade: The serine-threonine kinase Hippo (Hpo) which is activated by its adaptor protein Salvador (Sav), activates Warts (Wts) which associates with Mob-as-tumor-suppressor (Mats). Activation of this cascade leads to the phosphorylation of Yorkie (Yki), a transcriptional coactivator, responsible for the transcription of different genes involved in cell proliferation and prevention of apoptosis. This cascade is controlled by a number of upstream regulators, e.g. the proteins Expanded (Ex) and Merlin (Mer) which associate with the WW-domain protein KIBRA.

Previous experiments in our lab showed that Pals-1 Associated Tight Junction Protein (PATJ) is involved in the Hpo-Pathway in Drosophila. It was shown that PATJ is – in contrast to other components of the Crumbs complex - dispensable for cell polarity but regulates morphogenetic processes. PATJ plays a role in the accumulation of Myosin during the development of the adherens junctions and is implicated in metamorphosis.

Research Plan: Preliminary experiments showed that PATJ null mutants in flies exhibit a partial embryonic lethality which could be rescued by null mutations of the genes for the Hippo-Pathway upstream regulators KIBRA, Sav and Wts, a clear hint that PATJ is a possible upstream regulator of the Hpo-Pathway. Experiments have been done to show a principal biochemical interaction between PATJ and upstream Hpo-Pathway regulators Ex, Mer and KIBRA.

Recent publications have shown that Yki is not the only target of the Hpo-Pathway. For example activity of Enabled (Ena), an Actin Capping protein is also regulated by this kinase cascade through direct phosphorylation by Wts. Therefore, PATJ may not act through Ena instead of Yki. To test this hypothesis, experiments are presently performed to check if the PATJ mutation has any effect on the formation of the Actin- (Myosin-) Cytoskeleton. To test this hypothesis it will be checked if the migration speed of Drosophila Border Cells (small cell clusters in the ovaries which are wandering from anterior to posterior during oogenesis) is changed if a PATJ mutation is present. The migration speed is dependent upon correct formation of the Actin Cytoskeleton, if PATJ is interfering with Ena it should consequently alter the wandering ability.

Physiology and pathophysiology of ATPases in aldosterone secretion

Stindl J, Tauber P, Penton D, Sterner C, Tegtmeier I, Beuschlein F, Reincke M, Williams T, Mulatero P, Warth R, Bandulik S

Introduction: Aldosterone synthesis in adrenal glands is stimulated by angiotensin II and high plasma K^+. Both factors induce membrane depolarization, activation of voltage-gated Ca^{2+} channels, and an increase of cytosolic Ca^{2+}, which triggers the expression of aldosterone synthase. Autonomous renin-independent production of aldosterone, so-called primary aldosteronism (PA), is found in 10% of the patients with essential hypertension of which one third have aldosterone producing adenomas (APAs). Somatic mutations of the alpha subunit (ATP1A1) of the Na^+/K^+ ATPase are present in 5-6% of APAs. This study aimed at investigating the effects of mutants of ATP1A1 on intracellular Na^+ and pH homeostasis in adrenocortical cells.

Results: Primary cultured cells from patients with mutant $ATP1A1^{L104R}$ or $ATP1A1^{V332G}$ as well as NCI H295R cells expressing mutant $ATP1A1^{L104R}$ or $ATP1A1^{V332G}$ displayed a pathological membrane depolarization. In adrenocortical NCI-H295R cells expressing the mutated ATP1A1 (L104R or V332G), Na^+ extrusion was only reduced after blocking the endogenous ATPase. In addition, Fura-2 and BCECF fluorescence measurements showed that cytosolic Ca^{2+} signaling and pH balance was disturbed after expression of the mutant ATP1A1.

Discussion: These data indicate that the adenoma-associated mutants $ATP1A1^{L104R}$ and $ATP1A1^{V332G}$ result in a gain-of-function phenotype that disturbs intracellular electrolyte and pH homeostasis.

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Mutation of the Fanconi-associated protein 2 (FAP2) as a cause of hereditary renal tubulopathy

Julia Wiesner, Markus Reichold and Richard Warth

The metabolite 5'-methylthioadenosine (MTA) signals through the adenosine receptor A2B in melanoma

Katharina Limm and Anja Bosserhoff

Introduction: Recent studies revealed a tumor-supportive impact of loss MTAP expression in several kinds of tumors. In melanoma, the expression of MTAP is strongly reduced by promoter hypermethylation or genomic loss. MTAP converts the metabolite MTA to adenine and 5'-methylthioribose and thus combines the polyamine pathway and the methionine salvage pathway. Previously, we revealed that high levels of MTA outside the cells are associated with increased activity of the transcription factor AP-1, enhanced migratory potential of tumor cells, and promotion of tumor growth. MTA is also known as an inhibitor of protein arginine methyltransferase (PRMT) activity. It could be shown that high level of MTA resulted in a reduction the total protein methylation in melanoma cell lines and tissues. This could be linked to an additionally increased ERK activity. However, the molecular mechanisms of MTA-induced signaling are largely unknown.

Results: Adenosine receptors (ADORAs) were analyzed to be putative receptors for MTA signaling. The receptor ADORA A2B, one of the four known types of receptors, shows the strongest expression on mRNA level in melanoma cell lines. By stimulation of the G-Protein coupled receptor A2B modulation of the second messenger cAMP and the cAMP response element-binding protein (CREB) signaling pathway should be observed. Stimulation of the cells resulted neither in an increased cAMP level in the case of MTA nor in an activation of CREB pathway. These results indicate that stimulation of A2B did not activate the classical signaling cascade in melanoma cell lines. Instead we found a link between the A2B receptor and the activation of the transcription factor AP-1.

Discussion: Already in previous reports we could show that loss of MTAP expression has a profound impact on tumor cell activity in melanoma. We hypothesized that MTA itself has a tumor-promoting effect by stimulation of different tumor relevant signaling pathways. The ADORA family belongs to the G-protein-coupled receptor (GPCR) family, which consists of A1, A2A, A2B and A3. These receptors are of special interest, as they have been discussed as a potential target for melanoma therapy. We identified the adenosine receptor A2B as the receptor with the highest expression level in melanoma cells. Further we could show that MTA induced AP-1 activation occurs by stimulation of adenosine receptor A2B and activation of specific signaling pathways.

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Combining Computational Protein Design with Molecular Dynamics: A flexible approach to improve design accuracy

Patrick Löffler

Introduction: Protein interactions are of central importance for most biological functions. The ability to alter or even control protein interactions selectively would greatly advance applied biochemistry. In the past years the first reports on atomically accurate de novo designed interactions came up. The method of choice to generate such specific protein-protein or protein-ligand interactions at desired affinity, specificity and kinetics is computational protein design (CPD) coupled with high-throughput experimental characterization. However state-of-the-art CPD lacks precision and thus requires intensive biochemical optimizations to allow kinetics comparable to natural proteins. In order to increase the accuracy of CPD, the new approach discussed in the poster combines a classical rotamer optimization algorithm with Molecular Dynamics (MD) simulations.

Method: To design the binding of protein-protein interfaces or a protein binding site for a new ligand one generally starts from a given protein backbone and defined design spots. Based on an initial population of putative design sequences, this method explores the sequence space by rotamer optimization of each design sequence on a fixed protein backbone. In silico evolved design sequences are subsequently modeled with MD to allow a more detailed insight into their structural dynamics. After evaluating the structures generated by MD, reasonable structures with improved computed binding energies are selected to serve as the input for further sequence optimizations.

Proof-of-concept: In order to test the above mentioned method on a real world problem, a CPD goal was developed. Aminodeoxychorismate (ADC) synthases catalyze the chemical reaction of chorismate, a central metabolic intermediate, to 4-Aminodeoxychorismate. Another enzyme, ADC lyase catalyzes the cleavage of ADC to Aminobenzoate and pyruvate. Aminobenzoate then serves as a precursor for the important methyl-donor Tetrahydrofolate. A highly-similar enzyme named Anthranilate synthase (AS) also uses Chorismate as substrate but transforms it directly to Anthranilate and pyruvate, thus eliminating pyruvate in one step. As a proof-of-concept, the putative transition state for the pyruvate elimination in ADC synthases will be used as a design target to create de novo ADC synthases with elimination capabilities.

Interaction between osteoarthritic cartilage and chondrocytes/mesenchymal stem cells *in vitro*

Ute Mayer, Michaela Leyh, Joachim Grifka, Susanne Grässel

Introduction: Multipotent mesenchymal stem cells (MSC) bear the potential to be used in regenerative medicine, e.g. for cartilage repair after trauma. But not much is known about the capacity of MSC for cartilage regeneration under osteoarthritic (OA) conditions. To improve the quality of MSC-derived cartilage-like tissue a better understanding of the influence of an OA microenvironment including neighboring cells and tissue interfaces is necessary. Therefore, the interaction of OA cartilage and chondrocytes/chondrogenically differentiating MSC is investigated. Using an in vitro co-culture model, the influence of OA cartilage explants on co-cultured cells is analyzed regarding the expression of miR-124a, miR-675 and miR-29b which are associated with the regulation of Sox9 (chondrogenic master transcription factor), collagen II and I/III (ECM components), respectively.

Results: Chondrocytes/MSC embedded in fibrin gels and co-cultured on OA cartilage explants for 7 and 28 days in chondrogenic medium containing TGF-β show increased expression of miR-124a, miR-675 and miR-29b compared to monocultured cells in fibrin gels which served as controls. OA cartilage has no effect on Sox9 mRNA and protein level of co-cultured cells but leads to reduced collagen I, II and III mRNA and protein expression in chondrocytes/MSC.

Discussion: Increased miR-29b expression induced by co-culture with OA cartilage could explain reduced collagen I and III expression. To verify this, transfection experiments with miR-29b mimic and inhibitor are in preparation. miR-675 was identified as cartilage-specific, Sox9-dependent positive regulator of collagen II and Dudek et al. (J Biol Chem 2010, 285(32):24381–24387) suggested that miR-675 targets a collagen II transcriptional repressor. Therefore, reduced collagen II expression should be associated with reduced miR-675 and Sox9 expression. However, co-culture with OA cartilage leads to increased miR-675 expression in chondrocytes/MSC while Sox9 expression was not altered although the expression of miR-124a which targets Sox9 was increased. Thus miR-675 and miR-124a seem to target other mRNAs and reduced collagen II expression may be caused by other factors or other miRs.

The role of CMKLR1 and its ligands in non-alcoholic fatty liver disease

Rebekka Pohl, Sabrina Krautbauer, Kristina Eisinger, Michael Beck, Yvonne Hader and Christa Büchler

Estimating absolute abundances of bacteria in the intestinal microbiome

Frank Stämmler, Joachim Gläsner, Andreas Hiergeist, Udo Reischl, André Gessner and Rainer Spang

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The role of Syntrophin alpha and beta2 in Nonalcoholic Steatohepatitis (NASH)

Lisa Voggenreiter, Kristina Eisinger, Sabrina Krautbauer, Tobias Hebel and Christa Büchler

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