RIGeL Regensburg International Graduate School of Life Sciences

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BIOMEDIGS BIOMEDICAL INTERNATIONAL GRADUATE SCHOOL

ABSTRACTS

BIOMEDICAL SYMPOSIUM

for Graduate Stundents

8th and 9th November 2013

Universität Regensburg
FACULTY OF MEDICINE
FACULTY OF BIOLOGY

Herausgeber: Fakultät für Medizin
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BIOMEDICAL SYMPOSIUM

for Graduate Students

8th and 9th of November, 2013

Tagungshaus Bayerischer Wald, Regen

BIOMEDIGS
BIOMEDICAL INTERNATIONAL GRADUATE SCHOOL

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RIGeL Regensburg International Graduate School of Life Sciences

Organisation: BIOMEDIGS: Konstanze Bedal
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Fabian Herrmann
Janina Staffel
Julia Stindl
Philipp Tauber

PROGRAM Biomedical Symposium 2013

FRIDAY, 8th of November 2013

8:00 DEPARTURE from Klinikum bus stop (main entrance)

9:30-10:00 CHECK-IN at Tagungsghaus "Bayerischer Wald" in Regen

10:00-10:30 RECEPTION

10:30-12:00 **TALK SESSION 1** Chair: Carsten Broeker

1 **Metabolic characterisation of a-syntrophin knock out mice (SNTA -/-)**
Kristina Eisinger

2 **The role of Substance P and norepine-phrine in callus differentiation**
Tanja Niedermair

3 **Basophils as regulators of CD4+ T cell proliferation in a GvHD mouse model**
Fabian Herrmann

4 **Recruitment of CCR2+ monocytic myeloid cells by tumor-derived lactic acid in B16 melanoma**
Almut Brand

5 **The spatial energy expenditure configuration and possible applications in an experimental model of arthritis**
Susanne Klatt

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

12:00-13:00 LUNCH

13:00-13:30 Industrial Tutorial MLP

13:30-14:30 **POSTER SESSION 1**

1 **The Drosophila Nephrocyte – Ultrastructural and localization analysis of direct and indirect determining factors of cell polarity in null-mutant and knock-down flies**
Florian Hochapfel

2 **The Drosophila nephrocyte as a model for the podocyte**
Gudrun Mendl

3 **Can the cell type of renin expressing cells in the adult kidneybe programmed to an EPO producing cell type due to pVHL deletion?**
Katharina Gerl

4 **Renin cell lineage in the mouse kidney**
Ilona Schwarzensteiner

5 **Renal compensatory hypertrophy: Role of B-type natriuretic peptide**
Janina Staffel

6 **Physiology and pathophysiology of ion channels and ATPases in aldosterone secretion**
Julia Stindl

7 **Mutations of KCNJ5 K+ channel in aldosterone producing adenomas: pharmacology and calcium signaling in mutated cells**
Philipp Tauber

14:30-16:00 **TALK SESSION 2** Chair: Janina Staffel

7 **Regulation of the transcription factor c-Jun in malignant melanoma by the cytoskeleton**
Melanie Kappelmann

8 **Interaction of the tumor suppressor LKB1 with the membrane cytoskeleton**
Christian Thiele

9 **The role of PATJ in the development of Drosophila**
Arnab Sen

10 **Impact of methylthioadenosine (MTA) on cellular signaling**
Katharina Limm

11 **The WNT-pathway participates in the Osteogenic Differentiation of Human Dental Follicle Precursor Cells**
Sandra Viale-Bouroncle

12 **Collagen-induced arthritis modulates reactivity to sympathetic neurotransmitter stimuli during osteoclastogenesis of bone marrow-derived macrophages from DA rats**
Dominique Muschter

16:00-16:30 COFFEE BREAK

16:30-17:30 MEET AN EXPERT
Prof. Dr. Eberhard Hildt

18:00 DINNER

Metabolic characterisation of a-syntrophin knock out mice (SNTA -/-)

Kristina Eisinger, Sabrina Krautbauer, Yvonne Hader, Christa Buechler

The role of Substance P and norepinephrine in callus differentiation

Tanja Niedermair, Richard Stange, Britta Wieskötter, Anja Pasoldt, Rainer H. Straub, Andreas Zimmer, Joachim Straub, Susanne Grässel

INTRODUCTION:
During the progress of fracture healing bone and fracture callus become innervated by sensory and sympathetic nerve fibers. The absence of sensory innervation alters callus size and bone formation and may result in non-united fractures. Substance P (SP) is a sensory neuropeptide that is known to have an influence on cell proliferation, apoptosis, matrix formation and differentiation of mesenchymal callus tissue. Previous studies demonstrated that chondrocytes originating from callus tissue express SP and its receptor, neurokinin 1 (NK1-R). The catecholamine norepinephrine (NE) by contrast decreases apoptosis rate in vitro and seems not to be produced by chondrocytes in the callus. Aim of this research is to analyze the impact of SP and NE on callus differentiation and bone quality in a stabilized murine femur fracture model and an ex vivo fracture explant model.

RESULTS:
On day 5 after fracture the pressure sensibility in the fractured hind legs of Tac1-/- mice is reduced compared to WT mice, 8 days after fracture, the calli of Tac1-/- mice exhibit a lower volume and a higher density compared to fracture calli of WT mice. In explant cultures, gene expression of MIP2, cox2, timp-2, mmp-3, and mmp-14 is significantly higher in callus chondrocytes of Tac1-/- and sympathectomized mice than in chondrocytes of WT mice. 5 days after fracture sympathectomized mice do have a higher amount of mesenchymal callus tissue and a lower amount of soft callus tissue in the fracture callus compared to Tac1-/- and WT mice.Bone structure of Tac1-/- and sympathectomized mice is clearly altered compared to WT controls. 3 weeks after fracture, femora of WT mice resist stronger bending forces compared to Tac1-/- mice and resist to greater torque than femora of Tac1-/- and sympathectomized mice. The co-lateral control legs of WT mice resist to greater torque and are more stiff compared to Tac1-/- and sympathectomized mice. Quality of newly formed bone after fracture healing shows no difference compared to existing bone of co-lateral control legs.

DISCUSSION:
The absence of SP has an impact on pressure sensibility and on the expression of genes that play a role in inflammatory reactions and matrix composition. Absence of SP alters fracture callus size and density. NE and SP both affect the mechanical stability and quality of bone structure but they do not affect the quality of newly formed bone in adult mice after fracture healing.

Basophils as regulators of CD4+ T cell proliferation in a GvHD mouse model

Fabian Hermann, Manuel Rodriguez Gomez, Shazhad Sawas-Syed, Nicole Göbel, Yvonne Talke, Matthias Mack

INTRODUCTION:
T cell proliferation is an important part of immune regulation, as well as in malignant disease. We have found that non-activated, and activated basophils (DX5+,IgE+,FcεR1+) are able to inhibit T cell proliferation in vitro. This effect is independent of Fas and MHCII, but dependent on the soluble factors IL 4 and IL-6.

RESULTS:
We have found that non-activated and activated basophils (DX5+, IgE+, FcεR1+) are able to inhibit T cell proliferation in syngenic and allogenic MLRs in vitro. This effect is independent of Fas and MHCII, but dependent on the soluble factors IL 4 and IL-6. Based on these findings we analyzed the role of basophils in the model of GvHD and detected a significant increase in the GvHD score of mice after depletion of basophils. Adoptive transfer of basophils led to a significant reduction in the GvHD score compared to control. Mice depleted of basophils showed an increased CD4+ T cell count in spleen and mesenteric lymph nodes as well as an increase in total CD45+ cells in the GvHD model. Depletion of basophils also led to more IL-17+ CD4+ T cells in the mesenteric lymph nodes but not in the spleen compared to controls. To analyse the in vivo effects of IL-4 and IL-6 in the GvHD model we administered the cytokines or PBS. IL-4 treated mice showed a significant lower GvHD score and better survival compared to control mice. In contrast, administration of IL-6 significantly increased the GvHD score and reduced survival.

DISCUSSION:
These data suggest, that secretion of IL-4 and IL-6 by basophils might have beneficial as well as harmful effects on the outcome of a GvHD. There by basophils might be an interesting target to control an acute GvHD.

Recruitment of CCR2+ monocytic myeloid cells by tumor-derived lactic acid in B16 melanoma

Almut Brand, Katrin Singer, Gudrun Köhl, Maximilian Schmid, Uwe Ritter, Stefan Walenta, Wolfgang-Müller Klieser, Stefan Fichtner-Feigl, Matthias Mack, Marina Kreutz

INTRODUCTION:
The immunosuppressive microenvironment of tumors is caused by several factors, including cytokines and tumor metabolites like lactic acid. Lactic acid 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 monocytes to dendritic cells and the activation of T cells in vitro. The role of lactic acic in vivo will be illuminated in the following.

RESULTS:
Here we analyzed the impact of lactate on immune cell infiltration and tumor growth in a syngeneic mouse model. For this purpose we generated a stable murine B16.SIY melanoma cell line with low lactate secretion by means of Ldha short hairpin RNA technology. After s.c. application of 100 000 B16.SIYLdha-low (laclo) and negative control shRNA transfected B16.SIYLdha-high (lachi) melanoma cells, similar tumor growth occurred in immunodeficient RAG2-/- mice whereas tumor incidence and tumor growth of the laclo clone, compared to the lachi clone, was significantly delayed in immunocompetent C57BL/6 mice. This suggests that lactate modulates the immune response in C57BL/6 mice. Therefore we determined the immune cell infiltrate in laclo and lachi tumors and found no difference in tumor-infiltrating immune cells in RAG2-/- mice. In C57BL/6 mice lachi tumors exhibited significantly lower numbers of cytotoxic CD8+ T cells and increased numbers of CD11b+Gr-1+CCR2+ monocytic myeloid cells compared to laclo tumors. The recruitment of high amounts of monocytic myeloid cells is likely due to the accumulation of CCL2 in lachi tumors, which is associated with high lactate levels, as we could show. The immune escape of high lactate secreting tumors can be prevented by the depletion of CCR2+ cells, resulting in decreased tumor growth.

DISCUSSION:
We conclude that the CCR2/CCL2 chemokine pathway plays an import role in the recruitment of monocytic myeloid cells into tumor sites and that this process is modulated by lactic acid. Therefore pharmacological inhibitors of tumor cell glycolysis as well as anti-CCR2/CCL2 drugs could possibly not only target tumor cell growth directly but also support cancer immunotherapies.

The spatial energy expenditure configuration and possible applications in an experimental model of arthritis

Susanne Klatt, Rainer H. Straub

BACKGROUND:
Autoimmune responses 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. During 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 consumption of oxygen. The energy expenditure in different organs at different time points has never been investigated during immunization. We want to find out if, and how the energy expenditure in different organs changes during the course of experimental arthritis.

METHODS:
A new technique termed "spatial energy expenditure configuration (SEEC) is based on removal of tissue during the course of arthritis, and subsequent determination of oxygen consumption. Small pieces of the respective organ are placed in 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. 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.

RESULTS:
In draining lymph nodes of arthritic DBA/1J mice we observed a marked increase in oxygen consumption during the course of arthritis. Sympathectomy prior to immunization increases energy consumption in draining lymph nodes, which is most probably a sign of retention of leucocytes in the lymph node. C57BL/6 mice deficient for the important adipose triglyceride lipase revealed an increased oxygen consumption in the liver. This might be due to a lack of lipolysis activity, and therfore increased gluconeogenetic activity in the liver for the generation of energy rich fuels in form of glucose. ATGL-deficient arthritic animals also showed higher energy demand in lymph nodes, adrenals and gut.

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.

Creation and Characterization of Pkd2 Knock-in Mice

Denise Schmied, Karin Babinger, Ralph Witzgall

The Drosophila Nephrocyte - Ultrastructural and localization analysis of direct and indirect determining factors of cell polarity in null-mutant and knock-down flies

FlorianHochapfel, Michael Krahn

INTRODUCTION

Drosophila nephrocytes are podocyte-like mesodermal-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 anterior to the protoventriculus and along the dorsal heart, respectively. Their main function is the filtration of the haemolymph, 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. Nephrocytic over-expression of sns and its human homolog NPHS1 lead to very similar phenotypes. (Weavers et al., 2009).

RESULTS

We found that a null-mutation (Δ1) of the myosin regulating factor PATJ severely impairs the development of both filtration slits and channel networks. In L3 larvae, the overall number of diaphragms is reduced and the distinct transition between the channel layer and the intracellular area is lost. First results indicate that murine MUPP1, a PDZ domain containing protein similar to PATJ, which was shown to restore overall viability in PATJΔ1 homozygous mutant flies, is also able to achieve a full rescue of the nephrocytic phenotype. During differentiation, apico-basal polarity of epithelia is determined by localization of specialized proteins. In case of the podocytes, the sub-apical adherens junction migrates to the basal aspect. Upon formation of the foot processes, it colocalizes with the slit diaphragms, suggesting that so-called cell polarity determinants (CPD) play a role in podocyte differentiation. In nephrocytes, a knockdown of Bazooka (Baz) had severe effect on development. No slit diaphragms could be found. Stardust (Sdt) RNAi caused a mild effect with irregular diaphragms.

RESEARCH PLAN

By the help of laser confocal microscopy, we were able to roughly estimate the localization of a number of CPDs. In order to exceed resolution limitations, we will test fusion proteins with the miniSOG marker, a genetically encoded tag for correlated light and electron microscopy. We plan to assess the localization of endogenously expressed proteins, necessary for slit-diaphragm integrity and/or nephrocyte differentiation. So far we have cloned miniSOG constructs with mNephrin and Bazooka, which will be expressed specifically in nephrocytes.

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The Drosophila nephrocyte as a model for the podocyte

Gudrun Mendl, Michael Krahn

The nephrocyte, a distinct type of endothelial cells of Drosophila melanogaster, resembles in structure and function the podocyte of the mammalian kidney. Their main function is to remove harmful substances from the haemolymph and inactivate them by endocytosis and life-long storage. The Garland nephrocytes are located anterior to the protoventriculus and the pericardial nephrocytes can be found alongside the dorsal heart. Similar to the mammalian podocyte, the nephrocyte form a size- and charge-selective filtration slit diaphragm and develop a network of channels and lacunae in the cell periphery. A set of homologous proteins, which are involved in forming the characteristic structures of the nephrocyte and podocyte, respectively, predestine the fly model for basic kidney research.

Recently, it has been shown that RNAi knock-downs and null-mutations of cell polarity regulators have a strong effect on the correct development of the larval nephrocyte, manifesting in reduced numbers of filtration slits diaphragms and channel networks, respectively.

A main project of this thesis will be the establishment of functional screens to analyze the effect of cell polarity determinants, their downstream targets and upstream regulators on the nephrocyte. Currently we establish an in vivo assay, utilizing secreted fluorescent proteins of various sizes to visualize and quantify the uptake of waste into the L3 larval nephrocyte. Furthermore, we analyze the effect of mutations in the nephrocyte via toxin assays, feeding the larvae harmful substances (e.g. AgNO3, Lead acetate) and subsequently determining the lethality and life span in all developmental stages (larva, pupa, adult).

In regard to clinically relevant genes playing a role in kidney development/ diseases, we generate RNAi knock-downs of our genes of interest specifically in the nephrocytes. Further ultrastructural and functional analyses will provide more information about the significance of these proteins during development and maintenance of the function of nephrocytes (and thus podocytes).

Can the cell type of renin expressing cells in the adult kidneybe programmed to an EPO producing cell type due to pVHL deletion?

Katharina Gerl, Birgül Kurt, Armin Kurtz

pVHL-KO mice deficient for the von Hippel-Lindau protein (pVHL) in renin-expressing cells (Cre expression under the control of the endogenous renin-1d promoter) show some interesting abnormalities compared to control mice. In these mice the Vhl gene is already deleted during kidney development and Vhl deficiency consequently leads to a stabilization of the hypoxia inducible transcription factors (HIFs). The resulting phenotype is very interesting. On the one hand renin expression is diminished compared to control mice. Moreover, renin-expressing cells show an atypical localization: they are not located at the typical juxtaglomerular (JG) position at the entrance into the glomerulus, but they are arranged now at the branching sites to the interlobular arteries. On the other hand these mice were considerably polycythemic. This polycythemia is caused by an increased production of erythropoietin (EPO). In situ hybridization localized EPO mRNA expression at the JG positions of the afferent arterioles. This is very special, as at this site usually renin is expressed.

Based on these data we want to address, if renin cell specific deletion of pVHL in the adult stage causes a similar phenotype as seen in the pVHL-KO mice (with pVHL deletion occurring already during kidney development): diminished renin expression, atypical localization of renin-expressing cells and a phenotype shift to EPO producing cells. For this purpose we have two different mouse lines: the tamoxifen-inducible Ren1cCreER (from Kenneth W. Gross) and the doxycyclin-inducible LC1-mRenrtTAm2 (from Vladimir Todorov) mice. There is one more interesting mouse line, which enables us to delete Vhl in Cx40 expressing cells, namely the Cx40TAm-Cre (from Lucile Miquerol). Here it is interesting to investigate if HIF2α is stabilized in Cx40 expressing cells due to the deletion of Vhl and if this leads to an increased production of EPO in endothelial and intra- and extraglomerular mesangial cells in the kidney.

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Renin cell lineage in the mouse kidney

Ilona Schwarzensteiner, Birgül Kurt, Armin Kurtz

INTRODUCTION

Containing the key regulatory enzyme of the Renin-Angiotensin-Aldosterone-System (RAAS), renin-expressing cells are essential for the regulation of blood pressure and fluid-electrolyte homeostasis.

Although several studies have been performed to elucidate renin cell identity and maintenance, still little is known regarding renin cell precursors and cell deriving from renin expressing cells during kidney ontogeny. In order to track the renin cell lineage in aldosterone synthase knockout mice (Aldosynth-/-), we crossed in mice having the Cre-recombinase gene under the control of the endogenous renin promoter (Ren 1d-Cre) and a double fluorescent reporter (tdTomato/GFP). This reporter mouse expresses membrane-targeted tandem dimer tomato prior to Cre mediated excision and membrane-targeted green fluorescent protein after excision. We performed immunohistochemical studies on sections of paraffin embedded mouse kidney tissue to define the level of coexpression between several tissue specific markers and cells of the renin cell lineage (=GFP positive). Costaining was determined by counting cells positive for the marker as well as cells positive for both the marker and GFP.

RESULTS

Regarding the Aldosynth-/- mice we found 100% GFP staining of renin expressing cells and 60% staining of preglomerular arteriolar smooth muscle cells. A 60% staining was also seen in mesangial cells, whereas endothelial cells and podocytes remained negative. 20% of tubulointerstitial cells were positive for GFP. More than 80% of collecting duct cells were positive, whilst the proximal and distal tubule (including the macula densa) and thin limbs were negative for GFP. The medullary and cortical portions of the thick ascending limbs of Henle stained positive by 84% and 32%, respectively. We obtained similar results for the WT control, only the staining of preglomerular arteriolar smooth muscle cells showed a decrease to 40%.

DISCUSSION

These data suggest that a significant portion of preglomerular vascular smooth muscle cells, mesangial cells and also tubulointerstitial cells belong to the mesenchymal renin cell lineage having common ancestor cells. Apparently the renin promoter was active in the "Sammelrohranlage" at a given developmental stage. Interestingly, the renin promoter was also induced during development and differentiation of cells of the thick ascending loop of Henle, but not in other parts of the tubular system.

Renal compensatory hypertrophy: Role of B-type natriuretic peptide

Janina Staffel, Frank Schweda

INTRODUCTION:
Loss of kidney tissue, for instance due to unilateral uninephrectomy (UNx), results in a functional and morphological adaptation of the remaining intact nephrons. UNx in mice and living kidney donors induces a rapid increase in glomerular filtration rate (GFR) and also the growth of the remaining kidney tissue, especially of glomeruli and proximal tubules. The factor initiating this phenomenon, named renal compensatory hypertrophy, has not been yet identified. Our previous work has shown the guanylyl cyclase-A (GC-A) to play an important role in this process. GCA is the common receptor for natriuretic peptides ANP (Atrial natriuretic peptide) and BNP (B-type natriuretic peptide) that both lead to an increase in GFR. The aim of this study is to investigate which of these hormones triggers this rapid response after UNx.

RESULTS:
Three days after UNx (performed on mice) the glomerular filtration rate in the remaining kidney tissue increased to 140% to the basic value. During further observation GFR increased only slightly to 150%. We therefore conclude that the functional adaptation of the remaining kidney tissue occurs mainly within the first days after UNx. The plasma BNP concentration was significantly elevated in the UNx mice (111.0 + 14.8 pmol/L, sham 64.1 + 9.7 pmol/L, $p< 0.05$) three days post surgery, whereas the plasma ANP concentration was similar in both groups. Furthermore, urinary cGMP excretion was elevated, which matches the activation of GC-A. Physiological concentrations of both natriuretic peptides stimulated GFR and renal blood flow in isolated perfused mouse kidneys, with the vasorelexant effect of BNP being more pronounced than that of ANP. In fact, elevation of plasma BNP to post-UNx levels via osmotic minipumps for three days led to an increase in GFR by 20%.

DISCUSSION:
Three days after UNx plasma BNP levels are elevated and GFR and weight of the remaining kidney are markedly increased. BNP is a potent vasodilatator and stimulates the GFR in concentration dependent manner. BNP Infusion to post-UNx plasma levels markedly stimulates GFR and induces a slight kidney growth response. These data - together with our previous findings in GC-A knockout mice - indicate that the natriuretic peptide BNP plays a central role in renal compensatory hypertrophy by activating its receptor GC-A.

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Physiology and pathophysiology of ion channels and ATPases in aldosterone secretion

Julia Stindl, Sascha Bandulik, Richard Warth

Mutations of KCNJ5 K+ channel in aldosterone producing adenomas: pharmacology and calcium signaling in mutated cells

Philipp Tauber, David Penton, Ines Tegtmeier, Sascha Bandulik, Richard Warth

Regulation of the transcription factor c-Jun in malignant melanoma by the cytoskeleton

Melanie Kappelmann, Silke Kuphal, Anja-Katrin Bosserhoff

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INTRODUCTION

Malignant melanoma is an aggressive tumor derived from melanocytes. Recent studies have shown that c-Jun (member of the AP-1 transcription factor family) plays an important role in this disease. Recently we could reveal that c-Jun plays an important role in malignant melanoma. Previously we determined that the cytoskeleton seems to play an important role in c-Jun regulation and thus AP-1 activation, however, the precise role and the interaction with c-Jun stayed elusive.

RESULTS

Results of AP-1 activity measurements after Taxol and Nocodazol treatment suggested that the microtubule dynamics, and not the actin dynamics, are important for AP-1 activity. Moreover, decreased AP-1-DNA binding after Taxol and increased AP 1 DNA binding after Nocodazole treatment in melanoma cell lines could be detected. Due to the influence of microtubule disrupting agents (MTDs) on AP-1 activity we speculated c-Jun to be regulated by microtubule dynamics. Further experiments resulted in a decreased level of c-Jun protein in the nucleus after Taxol treatment whereas Nocodazole treatment accordingly led to a nuclear c-Jun accumulation. In addition, we detected a downregulation of AP-1 activity and a decreased c-Jun protein amount in the nuclear fraction after tubulin knockdown. Therefore, we speculated if tubulin stabilizes c-Jun protein in melanoma cells and thus influences c-Jun/ AP-1 activity. Furthermore, we demonstrated for the first time a direct interaction between tubulin and c-Jun by co-immunoprecipitation.

DISCUSSION

In summary, we could observe that microtubule dynamics significantly influence AP-1 activity in melanoma cells. Moreover, we could show that this influence on AP-1 activity by MTDs is c-Jun specific. Furthermore, we demonstrated for the first time a direct interaction between the transcription factor c-Jun and alpha-tubulin, which seems to stabilize c-Jun protein expression and thus AP-1 activity. Taken together these findings could possibly elucidate new regulatory mechanisms of c-Jun protein in malignant melanoma and thus lead to the identification of novel therapeutical targets.

Interaction of the tumor suppressor LKB1 with the membrane cytoskeleton

Christian Thiele, Michael P. Krahn

INTRODUCTION

LKB1 is a serine/threonine kinase that regulates diverse processes like cell proliferation, energy homeostasis and cell polarity. Using Drosophila melanogaster and other model organisms various downstream targets of LKB1 have been identified, while little is known about its upstream regulation.

To identify new potential interaction partners of LKB1 in Drosophila, a co-immunoprecipitation approach with subsequent mass-spectrometry was performed. In this study a subset of these candidates, which are components of the membrane cytoskeleton (α/β-spectrin and Lgl) and their functional relationship to LKB1 are being analyzed. The key questions are whether these proteins are involved in the localization of LKB1 or whether they are potential regulators and/or substrates of its kinase activity. This should further elucidate the role of LKB1 in cell polarity and proliferation control.

RESULTS

Immunohistological studies show that LKB1 is located at the basolateral cortex in embryonic and follicle cell epithelia, colocalizing with α/β-spectrin and Lgl, but no asymmetric distribution in embryonic neuronal stem cells (neuroblasts). To identify the domains of LKB1, which are important for this localization, GFP-LKB1 in S2R-cells has been analyzed by confocal fluorescence microscopy. Mutant constructs were transiently expressed, the membrane/cortex localization of LKB1 was observed to be dependent on farnesylation and a polybasic region near the C-terminus, similar to the mammalian protein K-Ras. In vitro experiments show a direct binding of this polybasic motif to certain phospholipids. Transgenic flies were constructed to investigate whether these effects are also valid in vivo. Rescue experiments of flies carrying a knockout allele of lkb1 show that the mutation of the polybasic region leads to a cytoplasmic localization and decreases viability. Co-immunoprecipitation using embryonic lysates shows a binding of the polybasic motif to α/β-spectrin. Clonal analysis of α-spectrin and β-spectrin indicate a role of β-spectrin but not α-spectrin for the localization of LKB1.

DISCUSSION

The cortical localization of LKB1 is depending on a lipid binding motif, which can bind to α/β-spectrin, both interactions are possibly involved in membrane targeting of LKB1. Since the cortical localization does not appear to be crucial for the function of LKB1 we are currently investigating the role of nuclear localization signals for its localization and function.

The role of PATJ in the development of Drosophila

Arnab Sen, Michael Krahn

INTRODUCTION

In epithelial cells a conserved set of proteins play an important role in establishing and maintaining cell polarity. One of the important protein complexes that are believed to be the key player in performing such a function is the Crb,Sdt,PATJ complex. (Crumbs-Stardust-Pals1 associated Tight Junction) (Assémat E et al. 2007). In this complex PATJ is supposed to form the third member of this complex and has been reported to play a crucial role for tight junction formation in mammalian cell (Shin K et al. 2005). In contrast little or contradicting evidences are known about the Drosophila homologue. In our current project we elucidate the roles of PATJ in developing and maintaining cell polarity and cell adhesion. The establishment and maintenance of cell polarity in epithelial cells is closely connected with the formation of cell-cell junctions. Notably, most of the key players regulating both processes are highly conserved throughout evolution, from worm to human. Cell-cell junctions need attachment to the actin-myosin cytoskeleton and we focused on non-muscle Myosin II.

RESULTS

In our present research we have identified the role of PATJ in cell polarity and maintaining stability in adherens junction in cell-cell contacts. Although PATJ have been reported to be the third component of the crumbs complex, deletion of the open reading frame of the protein in flies showed that PATJ is not per se crucial for the localization of other apical markers. In contrast interestingly PATJ have been found to play a regulating role in maintaining the stability of cell-cell junctions by modulating the phosphorylation of Myosin II. PATJ directly interacts with the Myosin Binding Subunit (MBS) of the Myosin Phosphatase and decreases Myosin dephosphorylation, resulting in activated Myosin dyanamics (Sen et al. 2012). Thereby PATJ supports the stability of the Zonula Adherens.

DISCUSSION

Since PATJ mutant shows minor defects regarding apical Actin-Myosin chain in Adherens Junction (AJ) stability we proposed that there can be some other AJ binding proteins which might have redundant functions in AJ stability along with PATJ. One such obvious protein is the Actin binding protein Vinculin. Although it have been shown before that Vinculin is not essential in flies, deletion of Vinculin along with PATJ completely destabilizes AJ and cytoskeletal architecture. Hence we hypothesize that PATJ and Vinculin may play redundant roles.

Impact of methylthioadenosine (MTA) on cellular signaling

Katharina Limm, Susanne Wallner, Anja-Katrin Bosserhoff

Methylthioadenosine phosphorylase (MTAP) is ubiquitously expressed in normal cells. In melanoma, the expression of MTAP is strongly reduced by promoter hypermethylation or genomic loss. It converts MTA to adenine and 5'-methylthioribose and thus combines the polyamine pathway and the methionine salvage pathway. Consequence of the loss of MTAP is an intra- and extracellular accumulation of MTA.

Previously, we revealed that high levels of MTA outside the cells are associated with increased activity of the transcriptional regulator AP-1 (activator protein 1), enhanced migratory potential of tumor cells, and promotion of tumor growth. MTA is also known as a regulator of protein arginine methyltransferase (PRMT) activity. We demonstrated that accumulation of MTA in melanoma leads to a reduction of total protein methylation in melanoma cell lines and tissues. This could be linked to an increased ERK activity. However, the molecular mechanisms of MTA-induced signaling are largely unknown. We aimed to define further signaling pathways which are regulated by extracellular MTA and focused on putative relevant receptors.

Adenosine receptors (ADORAs) may play a role in MTA signaling, because of the structural similarity of MTA to adenosine. We revealed that ADORA A2B, one of the four known types of receptors, shows the strongest expression on mRNA level in melanoma cell lines. Stimulation of G-Protein coupled receptors also has an effect on the regulation of the second messenger cAMP. Further, we revealed that cAMP is regulated by modulation of the Gs-coupled receptor upon treatment with MTA and a specific agonist of A2B (Bay60). To investigate the cAMP response element-binding protein (CREB) signaling pathway, we focused on the transcription factor cAMP response element (CRE). Stimulation of the cells resulted neither in an increased cAMP level in the case of MTA nor in an activation of CRE. Only a slight accumulation of cAMP occurred after Bay60 treatment. These results indicate that stimulation of A2B did not activate the classical CREB signaling cascade in melanoma cell lines. Instead we found a link between the A2B receptor and the activation of the transcription factor AP-1. Through investigation of different inhibitors for various signaling pathways we want to identify the complete signaling cascade of MTA mediated stimulation of A2B in melanoma cell lines. This might aid in tumor therapies based on metabolomic changes.

The WNT-pathway participates in the Osteogenic Differentiation of Human Dental Follicle Precursor Cells

Sandra D. Viale Bouroncle, Torsten Reichert, Martin Gosau, Christian Morsczeck

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Collagen-induced arthritis modulates reactivity to sympathetic neurotransmitter stimuli during osteoclastogenesis of bone marrow-derived macrophages from DA rats

Dominique Muschter, Nicole Schäfer, Rainer H. Straub, Joachim Grifka, Susanne Grässel

INTRODUCTION
Osteoclast (OC)-mediated bone destruction contributes to increased disease burden in Rheumatoid Arthritis. Simultaneously, changes in synovial tissue innervation occur, leading to a reduction in catecholaminergic nerve fibres. Studies on sweat gland innervation revealed that catecholaminergic fibres are capable of phenotypic transition to cholinergic nerves. The sympathetic neurotransmitters norepinephrine (NE), acetylcholine (ACh) and vasoactive intestinal peptide (VIP) affect osteoclastogenesis oppositely prompting us to study OC formation at different phases of collagen-induced arthritis (CIA) in an altered neurotransmitter microenvironment.
The influence of NE, ACh and VIP on differentiation and activity of bone marrow macrophage-derived osteoclasts from control and CIA animals are compared at various time-points post immunization (pI). The expression profile for NE, ACh and VIP receptors is analyzed on mRNA and protein levels.

RESULTS
ACh stimulation markedly elevated OC formation in controls (15 and 40 days pI). NE decreased osteoclastogenesis via beta-adrenoceptors and enhanced via alpha-adrenoceptor stimulation. VIP time-point dependently inhibited (10, 15 days pI) or stimulated (20, 40 days pI) osteoclastogenesis.Cells from arthritic animals were less affected. By trend, osteoclasts from arthritic animals showed decreased activity in a cathepsin K activity and in a matrix degradation assay.
Receptor gene expression changed in the time course of arthritis. 20 days pI muscarinic ACh receptors M3 and M5 were significantly upregulated whereas VIP receptor PACR1 was significantly downregulated. After 40 days adrenoceptors alpha1D and alpha2B were significantly downregulated. So far, on protein level we could not detect any CIA-induced changes.

DISCUSSION
We conclude that CIA partly suppresses osteoclast differentiation and activity as well as reactivity to neurotransmitter stimulation but the underlying processes remain unknown as yet. NE, ACh and VIP receptor expression was affected time-point dependently but the physiological impact still needs further investigation.

SATURDAY, 9th of November 2013

9:00-9:30 Meeting Biomedigs/RIGEL

9:30-10:45 **TALK SESSION 3** Chair: Fabian Herrmann

13 **Procollagen I-expressing renin cell precursors**
Christian Karger

14 **Pathomechanism of a novel form of renal Fanconi Syndrome**
Carsten Broeker

15 **New insights into the LMX1B target genes**
Natalya Stepanova

16 **Ultrastructural and cell biological studies of the primary cilium**
Benjamin Salecker

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

10:45-11:00 COFFEE BREAK

11:00- 12:00 **POSTER SESSION 2**

8 **Molecular function of the liver protein MIA2**
Mona Solanki

9 **Melanoma inhibitory activity (MIA) acts as a mediator of oncogene-induced senescence in human melanocytes**
Lena Honold

10 **Nuclear Magnetic Resonance Spectroscopy in the Study of Tumor Metabolism**
Jochen Hochrhein

11 **Predicting BV6 sensitivity in AML patients based on genome-wide gene expression profiles by integration of experimental and clinical data**
Anton Moll

12 **The influence of Collagen XVI on proliferation and invasion in oral squamous cell carcinoma**
Konstanze Bedal

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

12:00-13:00 LUNCH

13:00-13:45 Industrial Tutorial Promocell

13:45-14:45 **POSTER SESSION 3**

14 **p62 in death receptor signaling**
Kristina Seibold

15 **Characterization of expression and function of the bactericidal/permeability increasing protein (BPI) based on newly developed mouse models**
Nicole Bezold

16 **Functional analysis of bestrophin-1, an integral membrane protein of the retinal pigment epithelium associated with Morbus Best**
Sabrina Unkel

17 **Towards identifying the gene associated with North Carolina Macular Dystrophy in 6q14-16.2**
Karoline Wissmann

18 **Chronic psychosocial stress changes the activation state of T cells in a time – dependent manner**
Thi-Thu-Trang Nguyen

19 **Interaction between cartilage or subchondral bone and mesenchymal stem cells in vitro**
Ute Mayer

20 **A boolean approach for indirect inference of non-transcriptional pathways**
Martin Pirkl

21 **Stress-induced Glucocorticoids Mediate Re-cruitment of IMC into Sencodary Lymphoid Organs**
Dominic Schmidt

14:45-15:00 Coffee break + evaluation

15:00-15:30 Presentation Best Talk & Best Poster

16:00 DEPARTURE from Tagungshaus

Procollagen I-expressing renin cell precursors

Christian Karger, Armin Kurtz

INTRODUCTION
Renin-expressing cells in the kidney normally appear as mural cells of developing preglomerular vessels and finally impose as granulated juxtaglomerular cells in adult kidneys. The differentiation of renin-expressing cells from the metanephric mesenchyme in general and the potential role of special precursor stages in particular is not well understood. Therefore, it was the aim of this study to search for renin cell precursors in the kidney. As an experimental model, we used kidneys of aldosterone synthase-deficient mice, which display a prominent compensatory overproduction of renin cells that are arranged in multilayered perivascular cell clusters.

RESULTS
We found that the perivascular cell clusters contained two apparently distinct cell types, one staining positive for renin and another one staining positive for type I procollagen (PC1). It appeared as if PC1 and renin expression were inversely related at the cellular level. The proportion of renin-positive to PC1-positive cells in the clusters was inversely linked to the rate of salt intake, as was overall renin expression.

DISCUSSION
Our findings suggest that the cells in the perivascular cell clusters can reversibly switch between PC1 and renin expression and that PC1-expressing cells might be precursors of renin cells. A few of those PC1-positive cells were found also in adult wild-type kidneys in the juxtaglomerular lacis cell area, in which renin expression can be induced on demand.

Pathomechanism of a novel form of renal Fanconi Syndrome

Carsten Broeker, Markus Reichold, Nadine Assmann, Katja Dettmer, Kathrin Renner, Enriko Klootwijk, Jörg Reinders, Peter Oefner, Robert Kleta, Richard Warth

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New insights into the LMX1B target genes

Natalya Stepanova, 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 a rare autosomal-dominant disorder called nail-patella syndrome which affects limbs, eyes, brain and kidneys.

The binding of LMX1B to its target genes is mediated by the central homeodomain which specifically recognizes so-called FLAT (FAR linked AT-rich) elements. These elements can be subdivided into two versions: FLAT-E (5'-TAATTA-3') and FLAT-F (5'-TTAATA-3').

Microarray studies of glomeruli isolated from inducible podocyte-specific Lmx1b knock-out mice have shown a significant increase of 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. Two of them encode proteins associated with the actin cytoskeleton, they are Abra (Actin-binding Rho-activating protein) and Arl4c (ADP-ribosylation factor-like 4C). The third candidate of the studies, Crct1 (Cysteine-rich C-terminal 1), is currently biologically unexplored, making it attractive as a research object.

RESULTS

In HeLa cells LMX1B activates transcription of Firefly luciferase reporter driven by the human ABRA promoter (ABRA1). Moreover, transcriptional regulation depends on the presence of FLAT elements within promoter (ABRA1 vs. ABRA2 and ABRA3).

Abra showed strong co-localization with actin filaments in primary podocytes by immunofluorescence. Abra fused with MiniSOG confirmed these data by electron microscopy and demonstrate also a membrane-associated localization in finger-like protrusions of the cells.

Similarly to Abra, Arl4c and Crct1 showed membrane-associated localization at filopodia. However, Arl4c and Crct1 did not co-localize with F-actin.

Crct1, Abra and Arl4c lead to the increased formation of F-actin

DISCUSSION

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.

Ultrastructural and cell biological studies of the primary cilium

Benjamin Salecker, Cornelia Niemann, Helga Schmidt, Karin Schadendorf, Christine Meese, Sonja Gürster, Reinhard Rachel, Ralph Witzgall

INTRODUCTION

The non-motile primary cilium represents one kind of cilia on eukaryotic cells and can be found on most mammalian cells. It is thought to play a role as a signaling center during development and to act as a sensory organelle. In the kidney, the primary cilium is assumed to act as a sensor for measuring flow in renal tubules. The mechanical stimulus of its bending leads to the activation of a Ca2+-dependent messenger system. The primary cilium is composed of a basal body located near the cell surface and the ciliary shaft that extends into the extracellular space. The transition zone between the basal body and the shaft forms a diffusion barrier between the ciliary membrane and the somatic membrane. Mutations in genes encoding for ciliary proteins cause a variety of diseases, called ciliopathies. These 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 transmembrane protein 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. Seven of these BBS proteins form the BBSome, a cage-like protein complex that is involved in the intraflagellar transport (IFT).

RESULTS/DISCUSSION

We further investigate the ultrastructure of the primary cilium in 3D by means of electron tomography. The use of high-pressure freezing and freeze substitution during sample preparation results in the best structural preservation with less artifacts. 3D-models of the tomograms will be generated, yielding new insights in the ultrastructure of the primary cilium in 3D.

To answer the question of the subcellular distribution of ciliary proteins a genetically encoded tag for correlative light and electron microscopy, the so-called miniSOG protein, will be used for protein localization of polycystin-2, Septin-2 (which is said to contribute to the transition zone barrier) and the BBSome proteins. Together with electron tomography we aim to analyze the localization of cilium associated proteins in 3D.

We perform deciliation experiments with certain agents in order to synchronize the stage of ciliogenesis of cells in culture. Nocodazole treatment for example drives cells to lose their cilia and after a recovery time new cilia begin to emerge. This makes the investigation of different ciliogenesis stages with the EM controllable.

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

Baumgarten Susanne, Knoll Gertrud, Dueck Anne, Eichner Norbert, Naumann Uta, Englert Christoph, Meister Gunter, Witzgall Ralph, Zaparty Melanie

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

Podocytes represent an exceptional cell type with a complex cytoarchitecture which surround the capillaries of the renal glomerulus with their interdigitating foot processes. Since they play a crucial role in the renal filtration process, the aim of this work is the functional analysis of miRNAs important for their structure and function.

Recent studies using mice with a podocyte-specific deletion of Drosha and Dicer demonstrated the importance of miRNAs for development and maintainance of podocyte function (Harvey et al., Ho et al., Shi et al. 2008, Zhdanova et al. 2011). However, a possible (patho)physiological role in the kidney has been ascribed to only a few miRNAs so far.

We used double-fluorescent Cre-reporter mice to specifically enrich podocytes from isolated glomeruli by FACS (Boerries et al. 2013) and identify miRNAs by deep sequencing-analysis. For those miRNAs in silico predictions have been performed using ten commonly used algorithms to predict target mRNAs coding for important structural podocyte proteins. Validation of predicted miRNA-mRNA pairs was performed by Argonaute-immunoprecipitation (Beitzinger et al. 2007) using human podocytes. This technique will be transferred to freshly isolated mouse podocytes. Additionally, we perform luciferase assays to confirm predicted mRNA-miRNA pairs and the respective binding sites.

In order to manipulate endogenous miRNA levels, we applied two different techniques in vitro and in vivo. For a transient knock-down of a complete glomerular miRNA-family, zebrafish embryos were injected with Morpholinos. This treatment led to a strongly altered glomerular-specific phenotype (deformation, cyst formation). Currently, we investigate ultrastructural changes of the filtration barrier by electron microscopy. Additionally, we apply "miRNA sponges" representing competitive inhibitor transcripts that contain miRNA binding sites in their 3'-UTR (Ebert et al. 2007). The stable podocyte-specific in vivo expression of miRNA-sponges using lentiviral vectors is currently being established.

Molecular function of the liver protein MIA2

Mona Solanki, Anja K. Bosserhoff

The liver is the largest organ inside the human body and is responsible for various processes as 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 transported into the extracellular space by an N-terminal signal sequence. MIA2 belongs to the MIA gene family showing homology and sharing similarities in structure and genomic organization with the other members MIA, OTOR and TANGO.

MIA2 is highly overexpressed in patients with liver fibrosis and cirrhosis, leading to the conclusion that MIA2 expression responds to liver damage. Interestingly, the MIA2 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.

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 was further analyzed by co-immunoprecipitation. Moreover, the yeast-two-hybrid assay showed that another protein, the SH3-domain binding protein 1 might also interact with MIA2. This interaction will be further adressed in this work, too.

Finding interaction partners of MIA2 might give a deeper insight into the mode of action and molecular functioning of the protein.

Melanoma inhibitory activity (MIA) acts as a mediator of oncogene-induced senescence in human melanocytes

Lena Honold, Susanne Schiffner, Anja Bosserhoff

Malignant melanoma is a skin tumor which arises from the pigment-producing cells of the skin, the melanocytes. The protein melanoma inhibitory activity (MIA) has an influence on migration and invasion of melanoma cells, and thus plays a role in metastasis. We generated MIA deficient mice on the background of the Tg(Grm1)EPv transgenic melanoma mouse strain which is described to develop spontaneous melanomas with a short latency. Interestingly, EPv/MIA-/- mice showed an earlier onset of melanoma initiation compared to EPv/MIA wildtype animals. According to this, MIA also seems to be important in the early melanoma development in a protective way.

In contrast to normal human epidermal melanocytes (NHEM), melanoma cells secrete large amounts of the protein MIA. We observed that cultured primary NHEM show increasing MIA levels according to higher cell culture passages correlating with an increase in senescent cells. Therefore, we assume that MIA is involved in the induction of senescence in melanocytes.

Treatment of human primary melanocytes cultured at low passages with recombinant MIA led to an increased expression of the cell cycle inhibitors p16/INK4A and p21/CIP commonly known as senescence markers.

We recently described a MIA deficient melanoma cell line, HMB2-MIA. SA-ßGal positive cells could be found in the parental HMB2 cell line but not in MIA negative clones, further suggesting an impact of MIA on senescence induction.

To understand the regulatory pathways, reporter gene assays were performed after addition of recombinant MIA. We observed decreased AP-1 activity in different melanoma cell lines after addition of recombinant MIA. The transcription factor AP-1 has been shown to be a regulator of the cell cycle by acting as a repressor of tumor suppressor genes, such as p53, p21/CIP and p16/INK4A.

These data suggest a potential role of MIA in the induction of senescence via MIA-dependent gene regulation.

Nuclear Magnetic Resonance Spectroscopy in the Study of Tumor Metabolism

Jochen Hochrein, Eva Gottfried, Marina Kreutz, Oliver M. Grauer, Rainer Spang, Peter J. Oefner and Wolfram Gronwald

INTRODUCTION

Nuclear magnetic resonance (NMR) spectroscopy is a multifaceted platform for the investigation of tumor diseases using tissue extracts and biofluids. A single measurement of a sample yields a widespread overview of abundant small organic compounds (metabolites). Two different strategies of examination can be incorporated: targeted profiling and fingerprinting. In the targeted profiling approach a predefined set of metabolites is quantified while fingerprinting yields a snapshot of the whole small molecule (< 1 kDa) content of a given sample.

In the context of tumor metabolism two recent projects are described. The aim of the first project is the identification of metabolites potentially involved in immune escape mechanisms and thereby potentially promoting metastasis. In the second project the modulation of tumor metabolism by the non-steroidal anti-inflammatory drug diclofenac was investigated. NMR was applied to several different projects related to tumor metabolism, two of which are discussed in more detail.

RESULTS

With respect to the first project, we identified and quantified in a mouse model of breast cancer metabolites possibly taking part in immune escape mechanisms that spur metastasis. In the second project, where a murine glioma model was applied, we could show that the drug diclofenac modulated the metabolic state in a way that efficiently counteracted local immune suppression by substantially reducing lactate levels. Sample preparation, measurement and statistical data analysis were carried out according to established protocols.

Discussion

We successfully applied NMR spectroscopy to the study of tumor metabolism. Using targeted profiling as well as metabolic fingerprinting the metabolic state in different kinds of murine tissue was analyzed. We investigated the metabolic effects of treatment and mechanisms that spur metastatic spread.

Predicting BV6 sensitivity in AML patients based on genome-wide gene expression profiles by integration of experimental and clinical data

Anton Moll, Lars Bullinger and Claudio Lottaz

MOTIVATION:
BV6 is an antagonist of inhibitor of apoptosis (IAP) proteins. Since these are often over-expressed in cancer, BV6 may be used as a regulator of cancer cell survival in therapy. We predict BV6 sensitivity for patients by inference from experimental data to personalize therapy.

MATERIAL:
From 3 BV6-sensitive and 3 BV6-resistant patients of primary acute myeloid leukemia (AML), we take cells in culture to profile gene expression with Affymetrix microarrays (experimental dataset). As the clinical dataset, we use 93 AML cDNA-based patient gene expression profiles, published previously.

METHODS:
We limit both datasets to common genes and standardize expression values. Using Guided Clustering, we find gene sets which are differentially expressed between BV6-sensitive and BV6-resistant samples in the experimental dataset, and show similar correlation patterns in both datasets. To predict BV6-sensitivity of patients from the clinical dataset, we learn Support Vector Machines (SVM) from the experimental dataset limited to guiding genes.

RESULTS:
We obtained a gene set of 103 genes by Guided Clustering. A SVM based on this gene set predicts 55 patients as sensitive and 38 as resistant to BV6. According to Fisher tests, sensitive patients are strongly related to inv(16) (p-value 1.0e-14) and the FAB subtype 4 (p-value 2.8e-12). Noisy classifiers predict BV6-sensitivity of 78 of 93 patients similarly to the noiseless SVM.

CONCLUSION:
Our integrative approach has the potential to predict drug sensitivity of patients based on in vitro experiments, and thus to improve the personalized use of drugs

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

Konstanze Bedal, Susanne Grässel, Torsten Reichert, Richard Bauer

INTRODUCTION

Type XVI collagen (COLXVI) 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, COLXVI is important for the integrity of the basal membrane and the cross linking of elastic fibrils.

In preliminary works, we observed an overexpression of COLXVI and in oral squamous cell carcinoma (OSCC) patients (1,2,3). Overexpression of COLXVI in the human OSCC cell line PCI13 disclosed a positive impact on cell proliferation and invasion (2).On protein level we detected a strong influence of COLXVI on the activity of integrin beta 1 (ITGB1) and an increased expression of the integrin-associated protein Kindlin-1 in COLXVI overexpressing PCI13 cells. Moreover, the induction of COLXVI expression caused an increased expression of MMP-9 and invasion of COLXVI cells compared to mock control cells in collagen I gels

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RESULTS AND DISCUSSION

We demonstrate that Kindlin-1 interacts with the integrin-linked kinase (ILK) in COLXVI overexpressing PCI13 cells (COLXVI cells) in contrast to mock control cells. COLXVI cells show increased levels of phosphorylated and therefore activated ILK. In line with these data we observe higher amounts of phosphorylated protein kinase B (Akt/PKB) which is known to activate ILK by phosphorylation. The inhibition of ILK results in a decreased MMP-9 Expression on RNA level and protein level.

Moreover, MMP-9 promoter studies indicate a potential transcription factor (TF) binding site of the activator protein 1 (AP-1), as the inhibition of AP-1 via Tanshinone IIA results in a decreased MMP-9 expression on RNA and protein level. In addition, JunB, a member of the AP-1 family, showed differential expression between COLXVI cells and mock-control cells. These data indicate that Col XIV mediated ILK activation triggers MMP-9 gene expression via modulation of AP-1 in oral squamous cell carcinoma.

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

David Stieglitz, Anja K. Bosserhoff

Bone morphogenic protein6 (BMP6) is one of 20 known members of the BMP familiy and therefore belongs to the TGFβ superfamily of secreted growth factors. BMPs have been identified in various species with impact on a broad range of cellular processes, such as differentiation, proliferation, adhesion, motility and specification of cell fate during embryogenesis (Bragdon et al., 2011; Stuhlmiller & Garcia-Castro, 2012). They also play crucial roles during the development of organs and the nervous system and are controversially discussed referring to the formation and progression in different kinds of cancers.

In malignant melanoma increased expression patterns of BMP4 as well as BMP7 have been associated with enhanced migration and invasion of melanoma cells. Therefore underlying molecular regulatory mechanisms represent potential targets for prognosis and therapy of malignant melanoma.

Unpublished data also indicate a role of BMP6 during formation of malignant melanoma. The Bmp6-deficient Grm1-transgenic melanoma mouse line Tg(Grm1)/Bmp6-/- showed significantly decelerated melanoma development and progression of primary tumours compared to control mice. Interestingly enough, these findings are accompanied by an increased accumulation of dermal mast cells, indicating a potential role of the immune system in melanoma formation and progression.

The aim of this thesis is to elucidate the impact of BMP6 expression as well as the effect of mast cells on melanomagenesis.

p62 in death receptor signaling

Kristina Seibold, Wulf Schneider

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Characterization of expression and function of the bactericidal/ permeability increasing protein (BPI) based on newly developed mouse models

Nicole Bezold, Jens Wild, André Gessner

Human bactericidal/permeability increasing protein (BPI) is known for more than three decades to efficiently bind the lipid A region of lipopolysaccharide (LPS), triggering its opsonizing, microbicidal and endotoxin-neutralizing activities towards Gram-negative bacteria. As a protein with multifunctional roles BPI represents a central component of the innate immune system to directly combat microbes and modulate subsequent adaptive immune responses. Recently, the homologous protein of mice has been described, its expression and function are still ill-defined. New mouse models were developed to get a better insight in the regulation and function of BPI and its antagonist lipopolysaccharide binding protein (LBP), under homeostatic and inflammatory conditions. Initially BPI/LBP humanized mice were systematically characterized for human BPI and LBP expression on mRNA level, by quantitative real-time PCR analyses, as well as on protein level, by multiplex analyses and western blots. Furthermore the ability of in vitro stimulated granulocytes to release BPI was determined in this newly developed transgenic mouse model. To extend these studies immortalized bone marrow stem cell lines were generated by transduction with Hoxb8 expressing retroviruses. In order to monitor the bacterial burden, clinical course, and antibacterial immune response, BPI-deficient as well as the BPI/LBP humanized mice will be subjected to various in vivo infection models. To compare structure/function relationships, antibacterial spectra and efficacy between murine and human BPI, recombinant molecules were produced in Pichia pastoris and tested for their effects on different microbes.

Functional analysis of bestrophin-1, an integral membrane protein of the retinal pigment epithelium associated with Morbus Best

Sabrina Unkel, Bernhard Weber

Best vitelliform macular dystrophy (BMD) is a degenerative disease of the retinal pigment epithelium (RPE) with an autosomal dominant mode of inheritance. Characteristic for this disease are yolk-like lesions in the central area of the retina. Mutations in the gene BEST1 could be identified as cause of the disease and are also associated with several other types of retinal degeneration such as autosomal dominant vitreoretinochoroidopathy (ADVIRC), autosomal recessive bestrophinopathy (ARB) and adult-onset vitelliform macular dystrophy (AVMD). The encoded protein bestrophin-1 is an integral membrane protein, which is primarily expressed in the RPE where it localizes to the basolateral membrane. The function of bestrophin-1 is not yet completely determined. It is discussed whether it may function as an anion channel or as a regulator of the intracellular Ca2+ homeostasis. The effect of mutations on localization, processing and function of bestrophin-1 are not yet fully known either. This work therefore aims to at first analyze the localization of normal and mutant bestrophin-1 using stable transfected, polarized MDCKII cells to perform confocal microscopy and biotinylation assays. Furthermore the biochemical half-life time and the underlying degradation pathway (proteasomal, lysosomal or autophagy) will be assessed via a cycloheximide assay and use of specific inhibitors for the different degradation systems. In case mutant bestrophin-1 is degraded via the proteasomal pathway rescue experiments using chaperones could be investigated. In a next step the transport mechanism and proteins, which guide bestrophin-1 from the endoplasmic reticulum to the basolateral membrane of the RPE will be analyzed. Preliminary in silico studies could identify potential sorting signals within bestrophin-1. These sequence motives are generally involved in the transport of proteins to the basolateral membrane. By use of the p75 neurotrophin receptor we aim to investigate whether these potential sorting signals are able to cause a basolateral localization of bestrophin-1 in MDCKII cells. Finally, analysis on the potential function of bestrophin-1 as a chloride channel will be conducted with use of the ussing chamber. For this purpose stable with normal or mutant bestrophin-1 transfected cells will be stimulated by an apical osmotic shock and the chloride flux will be measured.

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Towards identifying the gene associated with North Carolina Macular Dystrophy in 6q14-16.2

Karoline Wissmann, Bernhard Weber

INTRODUCTION

North Carolina Macular Dystrophy (NCMD) is an autosomal dominant macular dystrophy showing variable expressivity but complete penetrance that rarely progresses. The clinical course can be classified into three different stages: the range from drusen-like lesions in the central macular at the level of the retinal pigment epithelium (grade 1) to larger lesions with confluent drusen in the retinal pigment epithelium (grade 2) to pigmentation on the edge of the lesion (grade 3). Following genetic linkage analysis, the gene for NCMD was mapped within chromosome 6 in q14-q16.2 and was named Macular Dystrophy, Retinal, 1 (MCDR1). Further refinement localized the gene to an interval of 1.8 Mb of DNA between D6S1716 to D6S1671. Sanger sequencing of all RefSeq protein-coding genes within the 6q14–q16.2 region of interest has not revealed NCMD-associated mutations. The aim of the work is identifying the genetic defect in NCMD.

RESULTS

The entire candidate interval was analyzed in two affected patients (mother and daughter) and the unaffected father by next generation sequencing. To reveal NCMD-associated candidate variants bioinformatic evaluation and sophisticated filtering of the raw data were performed and verified by Sanger sequencing. Further, potential disease causing transcripts were amplified in several human tissues and retinal cell culture by RT-PCR. Next generation sequencing and verification by Sanger sequencing on genomic level revealed 13 unique candidate variants. They range from single base pair substitutions to deletions and insertions of up to twenty base pairs. Mapping these sequence variants to the genomic sequences on chromosome 6, no variant was localized to a protein coding gene sequence. Although, we could show that five variants localized to intronic regions of a yet unknown spliced gene were highly expressed in human retina and brain. Full length sequence of the transcript and its gene structure were identified by RACE-analysis.

DISCUSSION

These findings suggest that untranslated gene regions, intronic sequences or non coding elements at the 6q14-q16.2 locus are likely candidates for NCMD-causing effects. This may provide insight into the role of the newly discovered gene in the pathogenesis of macular dystrophy.

Chronic psychosocial stress changes the activation state of T cells in a time – dependent manner

Trang Thi Thu Nguyen, Dominic Schmidt, Nicole Uschold, Daniel Peterlik, Daniela N. Männel, Stefan O. Reber, Anja Lechner

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Interaction between cartilage or subchondral bone and mesenchymal stem cells in vitro

Ute Mayer, Michaela Leyh, Joachim Grifka, Susanne Grässel

INTRODUCTION

Osteoarthritis (OA), a degenerative joint disease which mainly affects hands, knees, hips and spine, is characterized by pain, movement impairments and irreversible cartilage degradation. Age, obesity, sex and genetic predisposition are risk factors to develop OA (1, 2, 3). To treat OA stem cell-based strategies are promising because adult mesenchymal stem cells (MSC) show chondrogenic differentiation in vitro when treated with growth factors like BMPs or TGF-β (4). But ex vivo production of tissue-engineered cartilage and in situ induction of cartilage regeneration remains difficult. To improve the quality of MSC-derived cartilage-like tissue a better understanding of influences of the OA microenvironment like neighboring cells and tissue interfaces is necessary in which the differentiation of implanted MSC should take place in vivo.

RESEARCH PLAN

Aim of the project is to analyze influences of OA-cartilage and subchondral bone on chondrogenic differentiation of MSC in a co-culture in vitro model. Therefore MSC, chondrocytes or a mix of both cell types are embedded in fibrin gels and co-cultured on cartilage or subchondral bone explants and in monoculture as controls. To explore the question if cartilage and subchondral bone have an effect on microRNA (miR) expression of co-cultured cells TaqMan qPCR will be performed to have a look at expression of miR-29b, -675, -29a and -124 which are associated with collagen I (5), II (6), III (7) and Sox9 (8), respectively. Another question is if co-cultured cells alter protein expression of OA-cartilage. Therefore a protein isolation protocol for co-cultured and control cartilage explants has to be established and if differently expressed protein bands appear in Coomassie Blue and/or silver stained SDS-PAGE gels proteins will be identified by MALDI-TOF. Moreover a multiplex immunoassay is planned to compare secreted cytokines of OA- and healthy cartilage like interferons, interleukins, colony-stimulating factors, tumor necrosis factors, growth factors and chemokines.

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A boolean approach for indirect inference of non-transcriptional pathways

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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) proposed a method for updating pathway models (prior knowledge models PKN) in the light of new data from perturbation experiments. They define a Boolean network on the topology of a prior knowledge network and update those logical networks using phosphorylation assay data observed after perturbing multiple pathway molecules alone and in combination. Network updating is achieved by minimizing an objective function that combines the mean squared error and a penalty for network size. Edges from the PKN are either removed or equipped with logical functions. The network can thus distinguish between cooperative activation of a molecule by multiple incomming signals and alternative activation by these signals. Besides activation, signaling inhibition can also be modeled.

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.

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We propose a hybrid model that combines features of boolean networks and nested effects models allowing for indirect estimation of signaling pathway logics from downstream effects in gene expression.

Stress-induced Glucocorticoids Mediate Recruitment of IMC into Sencodary Lymphoid Organs

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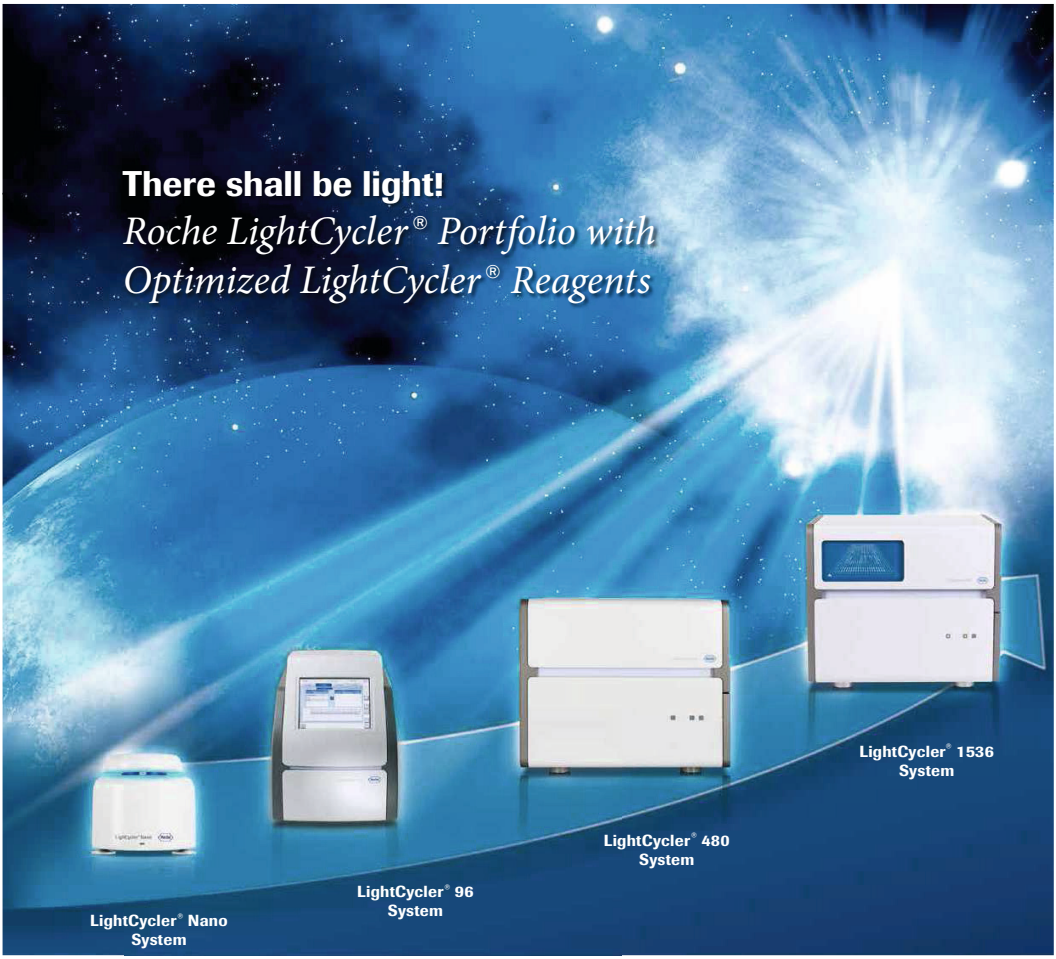
Chronic psychosocial stress has long been recognized as a risk factor for various immunological disorders. In a physiological stress response glucocorticoids (GC) are released out of the adrenals to prepare the body for a fight-or-flight response. In contrast to the activating, acute effects, chronically elevated levels of GC lead to immune suppression, specifically in inhibition of T- and B-cell function. Newer data indicate that GC have an activating effect on myeloid cells. The chronic subordinate colony housing (CSC) paradigm is an established model for chronic psycho-social stress and in the present study we investigated the recruitment of CD11b+ cells into secondary lymphoid organs. We therefore applied 19d of chronic stress to mice and detected an increased number of CD11b+ Ly6G+ Ly6Cint polymorpho-nuclear cells (PMN) as well as CD11b+ Ly6G- Ly6Chigh monocytic myeloid cells (MO) in the spleen, which in the literature are known as inflammatory myeloid cells (IMC). Stress-induced IMC produced more pro-inflammatory cytokines upon LPS stimulation. In the very early phase of the CSC paradigm we detected highly elevated levels of G-CSF in the serum indicating an increased haematopoiesis. An increased expression of chemokines in the spleen after 19d of CSC stress mediates a long-lasting recruitment of IMC into the spleen. In order to prove that GC mediate the stress-induced alterations, we injected dexamethasone into naïve mice and observed the same effects.

In conclusion our data indicate, that chronic stress induces an increased haematopoiesis of myeloid cells and a long lasting recruitment of IMC into the spleen, which produce high levels of pro-inflammatory cytokines.



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