HEINRICH HEINE
UNIVERSITÄT DÜSSELDORF

BMFZ

8. BMFZ-Klausurtagung
Tagungshotel „Maria in der Aue“
in Wermelskirchen
5. – 6. September 2013

Inhalt Seite

HEINRICH HEINE
UNIVERSITÄT DÜSSELDORF

BMFZ

8. BMFZ-KLAUSURTAGUNG
Tagungshotel „Maria in der Aue“ in Wermelskirchen
5. – 6. September 2013

Programm

Donnerstag, 5. September 2013

14.00 h	**Begrüßung**	**Guido Reifenberger**
14.15 h - 16.00 h	**Sitzung 1**	**Moderation: Sascha Weggen**
	Identification and characterization of regeneration-associated genes (RAGs) by paradigm-specific gene expression profiling of injured PNS	Frank Bosse, Hans Werner Müller, Molekulare Neurobiologie
	Autoimmune encephalopathies: Expression of patient derived antineuronal antibodies in recombinant form and analysis of their properties & role in pathogenesis	Manish Malviya, Norbert Goebels, Neurologie
	Generation of a faithful model for familial Alzheimer’s disease with presenilin-1 mutations by dual recombinase-mediated cassette exchange	Sascha Weggen, Neuropathologie
	The tgDimer mouse – a novel model for early events of Alzheimer´s disease	Andreas Müller-Schiffmann, Carsten Korth, Neuropathologie
	Dopamine regulation by functional DISC1 aggregates	Verian Bader, Carsten Korth, Neuropathologie
	Bioinformatic and biostatistical consulting and services for the BMFZ	Holger Schwender, Zentralbereich Bioinformatik u. Biostatiskik, BMFZ
	The role of EGFR-signalling in cutaneous homeostasis and immunity	Bernhard Homey, Hautklinik
16.00 h – 16.30 h	**Kaffeepause**	
16.30 h – 18.15 h	**Sitzung 2**	**Moderation: Heiner Schaal**
	Early infection dynamics of *Trichomonas vaginalis*	Gary Kusdian, Bill Martin, Molekulare Evolution
	The lymphotoxin-β receptor (LTβR) and its role in hepatocyte-mediated liver regeneration	Ursula Sorg, Klaus Pfeffer, Medizinische Mikrobiologie und Krankenhaushygiene
	Msb2 of *Candida albicans*: Environmental sensor and protectant	Mark Swidergall, Joachim Ernst, Mikrobiologie
	CaDom34: more than a quality control in nogo decay	René Geißen, Joachim Ernst, Molekulare Mykologie
	HEXploring of the HIV-1 genome allows landscaping of new potential splicing regulatory elements	Heiner Schaal, Virologie
	Quantitation of single protein aggregates for diagnostics of protein misfolding diseases	Oliver Bannach, Dieter Willbold, Physikalische Biologie
	Non-conventional approaches for structural and functional studies of membrane proteins	Manuel Etzkorn, Dieter Willbold, Physikalische Biologie
19.00 h	**Gemeinsames Abendessen, anschließend: Individuelle Diskussionsrunden im Biergarten**	

HEINRICH HEINE UNIVERSITÄT DÜSSELDORF

BMFZ

6. BMFZ-KLAUSURTAGUNG
Tagungshotel „Maria in der Aue“ in Wermelskirchen
5. – 6. September 2013
Programm

Freitag, 6. September 2013

8.00 h – 9.00 h	**Gemeinsames Frühstück**	
9.15 h – 10.45 h	**Sitzung 3**	**Moderation: Markus Uhrberg**
	Bacterial expression of p35 as a novel strategy to dissect IL-12 and IL-35 functions	Jens Moll, Jürgen Scheller, Biochemie u. Molekularbiologie II
	The F_o subcomplex of chloroplast ATP synthase	Daniel Schlieper, Georg Groth, Biochemische Pflanzenphysiologie
	Quantitative protein mass spectrometry	Kai Stühler, Molecular Proteomics Laboratory (MPL), BMFZ
	Next Generation DNA Sequencing: Strategies for targeted resequencing	René Deenen, Stefanie Stepanow, Karl Köhrer, Genomics & Transcriptomics Laboratory (GTL), BMFZ
	Phenotypic and functional NK cell deficiencies in patients with Myelodysplastic Syndrome	Maryam Hejazi, Markus Uhrberg, Transplantationsdiagnostik und Zelltherapeutika
10.45 h – 11.15 h	**Kaffeepause**	
11.15 h – 12.45 h	**Sitzung 4**	**Moderation: Friederich Boege**
	Ageing perturbs clock gene dependent regulation of autophagy in human skin	Hans Reinke, Friederich Boege, Klin. Chem. u. Laboratoriumsdiagn.
	Role of the Ulk1-Atg13-FIP200-Atg101 Complex in the Regulation of Autophagy: Crosstalk, Shortcuts, and Feedbacks	Björn Storck, Sebastian Wesselborg, Molekulare Medizin
	Possible role of PGRMC1 in breast cancer development	Hans Neubauer, Tanja Fehm, Frauenklinik
	Establishment of an automated high-throughput functional screening of the microRNAome in glioma cells	Bastian Malzkorn, Ranja Wader, Guido Reifenberger, Neuropathologie
	Dezellularisiertes, porcines Tränendrüsengewebe als Matrix für die Tränendrüsenregeneration	Kristina Spaniol, Stefan Schrader, Gerd Geerling, Augenklinik
	Keratin films for ocular surface reconstructions	Maria Borelli, Stefan Schrader, Gerd Geerling, Augenklinik
13.00 h - 14.00 h	**Gemeinsames Mittagessen**	
	Ende der Klausurtagung	

2. Abstracts

AG Fritz Boege

Ageing perturbs clock gene dependent regulation of autophagy in human skin

Hans Reinke, Fritz Boege
Klinische Chemie und Laboratoriumsdiagnostik

Autophagy is an intracellular breakdown mechanism that counteracts chronic organ degeneration and supports a prolonged life span by clearing defective macromolecular protein complexes and dysfunctional mitochondria. Rhythmic induction of autophagic flux by the circadian clock through the transcriptional induction of key autophagy genes has been demonstrated in liver cells and is considered to contribute to the synchronization of the cellular metabolism to feeding-fasting rhythms. Accumulating evidence suggests that the mammalian ageing process leads to a decrease in autophagy activity as well as circadian rhythmicity indicating that the circadian clock might also regulate autophagy levels during ageing. In this study we addressed the circadian control of autophagy and its decline upon ageing in cellular models for circadian rhythmicity and in aged primary human fibroblasts. Transition of fibroblast cells into the stationary phase induced an increase in autophagy gene expression concomitantly with transcriptional up-regulation of the core clock gene Per2. In stationary cells autophagic flux levels were highly rhythmic and genetic disruption of the circadian clock through knockdown of Bmal1 resulted in loss of rhythmic autophagy activity. Also in clock-less Cry1/Cry2-deficient fibroblasts up-regulation of Per2 was correlated with an increase in autophagy and autophagy gene expression. Interestingly, human dermal fibroblasts aged in situ likewise display a strong correlation between Per2 expression and autophagy activity suggesting that altered core clock gene regulation might contribute to the age-related decrease of autophagy in human skin cells.

AG Joachim Ernst

1. CaDom34 more than a quality control in nogo decay

René Geißen, Joachim Ernst
Molekulare Mykologie

Dom34 is a protein that is involved in no go decay in eukaryotic translation. This describes a process in which translation is stalled due to structured or misfolded mRNA. It is assumed that Dom34 is able to release blocked ribosomes from those mRNAs via RNAse activity together with the protein Hbs1. Characteristic for Dom34 is a so called SM-fold domain. SM-fold proteins are often involved in RNA metabolism, including degradation, RNA-RNA interaction or RNA-protein interaction. Previous studies in our group indicate furthermore that it has specific effects on translation of genes that are important for mannosylation of proteins in *C. albicans*, the so called protein mannosyltransferases (*PMT* genes), especially *PMT1*. *PMT* genes are essential for O-glycosylation of proteins and thus important for cell wall composition. On the one hand overexpression of Dom34 is sufficient to repress a *pmt1* phenotype and additionally it has been observed that protein amount of Pmt1 is increased while the mRNA levels stay constant. First *in vitro* studies show binding of Dom34 to short RNA oligonucleotides that originate from the 5‘-UTR of *PMT1* and it seems that a CAACCA motif is important for this binding.
In this study binding of Dom34 to untranslated (UTR) regions of *PMT1* and effects on translation will be analyzed *in vitro*. For this purpose an *in vitro* transcription system will be established to generate transcripts that can be used for binding analysis and translation with a eukaryotic translation system. Our findings show that Dom34 is able to bind to the 5’-UTR of *PMT1* and as a consequence translation of a reporter gene is influenced. Additionally the results indicate that Dom34 has an RNAse activity itself. If this is the reason for the effects on translation or additional activity of this SM-fold protein are responsible remains to be resolved.

2. Msb2 of *Candida albicans*: Environmental sensor and protectant

Marc Swidergall ,Joachim Ernst
Molekulare Mykologie

The yeast *Candida albicans* is commonly found as a commensal on the surface of human mucosa, but it can behave as an opportunistic pathogen when host immune defenses are low. Its capacity to change morphology between a yeast and a hyphal growth form is crucial for its pathogenicity and allows the formation of biofilms. Microbial biofilms are defined as structured microbial communities of single or multiple microbial on surfaces. Biofilm-embedded organisms exhibit drastically increased resistance to antimicrobial therapy and escape host immune defenses. Immune cells detect surface structures of *C. albicans* including glucan and mannoproteins and trigger response mechanisms including the production of antimicrobial peptides (AMPs). AMPs kill the pathogen and attract immune cells.

In *C. albicans* the glycoprotein Msb2 in the plasma membrane is a key signaling element in responses to defects in the cell wall of *C. albicans* that occur under immune attack or by treatment with antifungals. Msb2 is efficiently cleaved during growth and the extracellular glycodomain is quantitatively shed into the growth medium. Interestingly, the shed extracellular domain of Msb2 effectively protects fungal and bacterial cells from the action of AMPs. This inactivation of AMPs depends on binding to the shed glycofragment. In addition, the secreted Msb2 inactivates the lipopeptide antibiotic daptomycin, which is of special importance in current anti-infectious therapy because it serves as reserve antibiotic for the treatment of multiresistant Gram-positive bacteria. Thus, in addition to its sensor function, Msb2 has a second role in generating AMP resistance by shedding its glycofragment. Mixed infections of *S. aureus* and other important bacterial pathogens with *C. albicans* are of particular risk because bacteria are cross-protected by the shed fungal Msb2 protein against peptide antimicrobials.

AG Tanja Fehm

Possible role of PGRMC1 in breast cancer development

Hans Neubauer, H. Schneck, Tanja Fehm
University Women's Hospital

Hormone therapy may increase the risk of breast cancer. Thus, especially the addition of synthetic progestins may play a decisive role according to the results of clinical studies. Overexpression of a special receptor, i.e. the progesterone receptor membrane component-1 (PGRMC1), may offer a potential new pathway to explain the observed increase in breast cancer risk in the combined arm of the Women's Health Initiative. PGRMC1 is expressed in breast cancer tissue and may be important in tumorigenesis. The expression of PGRMC1 in breast cancer tissue is significantly different from that in normal mammary glands. Certain synthetic progestins can increase the proliferation of PGRMC1-overexpressing breast cancer cells and may thus be involved in tumorigenesis, while progesterone and certain synthetic progestins such as nomegestrol or chlormadinone acetate react neutrally. Our investigations point towards an important role of estrogen receptor-α in the signaling cascade, resulting in the proliferative effect induced by progestins. Thus, activation of PGRMC1 may explain the increased breast cancer risk observed during treatment with certain progestins. Very recently, PGRMC1 was investigated in serum samples of lung cancer patients and matched healthy patients; significantly higher concentrations were shown in the cancer patients. Therefore, PGRMC1 might be a predictor for other cancers as well but, according to clinical trials, its importance for a possible screening tool, particularly for breast cancer risk during hormone therapy, seems of interest.

AG Charlotte von Gall

NOD mice show altered daytime behavior and changed suprachiasmatic nucleus activity after manifestation of diabetes

Marc Ingenwerth[1], Anna Lena Reinbeck[2], Gilles Séquaris[2], Anna Stahr[1], Hans-Joachim Partke[2], Volker Burkart[2], Michael Roden[2,3], Charlotte von Gall[1]
[1]Anatomy II, [2]Clinical Diabetology, German Diabetes Center, [3]Endocrinology and Diabetology

The mammalian circadian pacemaker is located in the suprachiasmatic nucleus (SCN) of the hypothalamus and controls circadian rhythms in the sleep wake cycle, hormone production, food intake and immune as well as metabolic functions. The molecular clockwork within the SCN is based on transcriptional/translational feedback loops of clock genes. Disruption of the circadian clock is associated with metabolic dysfunction. The pathogenesis of type 1 diabetes is based on autoimmune processes, which ultimately leading to insulinopenia with hyperglycemia but may also affect insulin action. We tested the hypothesis that during the onset of diabetes daily rhythms in behavior and metabolism are disrupted and SCN activity is changed.

Female C57BL6, normoglycemic and newly diabetic (<3 d) NOD mice were kept at a 12-h light/12-h dark cycle. Respiratory quotients (RQ) were determined by indirect calorimetry. Locomotor activity was monitored by interruption of infrared beams (“counts”). Expression of the transcription factor cFos, reflecting neuronal activity in the SCN, was analyzed by immunohistochemistry.

In light cycles all mice show comparable levels of spontaneous locomotor activity, whereas in dark cycles diabetic NOD mice have a lower locomotor activity (575±84 counts) than C57BL6 (1164±478, counts, $p<0.05$) or nondiabetic NOD mice (1169±299 counts, $p<0.05$). Furthermore, diabetic NOD mice have a reduced RQ in light (0.82±0.02) and dark cycles (0.84±0.01), when compared to C57BL6 (light 0.88±0.01, dark 0.94±0.02, $p<0.001$) and nondiabetic NOD mice (light 0.88±0.02, dark 0.95±0.01, $p<0.001$). cFos expression was about 40% higher ($p<0.05$) in the SCN of C57BL6 and nondiabetic NOD mice at ZT02 (2 h after lights-on) than at ZT14 (2 h after lights-off). In contrast, diabetic NOD mice showed equal cFos expression at both time points (cFos positive cells in SCN: ZT02: 73±17, ZT14: 78±16).

In conclusion, newly developed type 1 diabetes is associated with altered daily pattern of behavior and metabolic function as well as changes in SCN neuronal function.

AG Gerd Geerling

1. Dezellularisiertes, porcines Tränendrüsengewebe als Matrix für die Tränendrüsenregeneration

Kristina Spaniol, A. Kunze, Gerd Geerling, M. Metzger, Stefan Schrader, Augenklinik

Fragestellung: Ein Tränendrüsenersatz auf der Basis von in vitro kultiviertem Tränendrüsengewebe stellt eine mögliche zukünftige Therapieoption zur Behandlung der schweren Tränendrüseninsuffizienz dar. Ziel dieser Arbeit war es, die Struktur und wesentliche Basalmembranbestandteile der nativen und der dezellularisierten porcinen Tränendrüse zu ^^vergleichen um zu untersuchen, ob dezellularisierte Tränendrüsenmatrices als Gerüst für die Rezellarisierung und damit zur in vitro Regeneration von Tränendrüsengewebe genutzt werden können.

Methodik: Für die Untersuchungen wurde Tränendrüsengewebe von sechs Hausschweinen genutzt (n=6). Jede Drüse wurde in vier Teile geteilt. Zwei Teile wurden nativ belassen, zwei Teile wurden dezellularisiert. Für die Dezellularisierung wurde die Drüse in ca. 3x3 mm große Stücke zerkleinert und über Nacht in Natrium-Desoxychelat in ultra-reinem Wasser inkubiert. Die Gewebestücke (nativ und dezellularisiert) wurden in Paraffin und OCT (optimum cutting temperature compound) eingebettet und nach H&E Färbung histologisch untersucht. Zusätzlich wurde die Expression von Basalmembranmarkern wie Laminin, Kollagen IV und Fibronektin immunhistochemisch evaluiert.

Ergebnisse: Histologisch zeigte sich eine intakte Struktur der extrazellulären Matrix nach Dezellularisierung des Tränendrüsengewebes. Über die Kernfärbung mit DAPI wurde der Verlust der Kerne und somit die erfolgreiche Dezellularisierung nachgewiesen. Die Basalmembrankomponenten Kollagen IV und Laminin waren immunhistochemisch vor und nach Dezellularisierung bandförmig im Bereich der Basalmembran nachweisbar.

Schlussfolgerungen: Nach Dezellularisierung von porcinem Tränendrüsengewebe zeigte sich ein intaktes Gewebegerüst der Tränendrüse, mit überwiegendem Erhalt der extrazellulären Bestandteile der Acini. Immunhistochemisch konnte ein Erhalt von Basalmembranstrukturen wie Kollagen IV und Laminin nachgewiesen werden. Ein Erhalt der Basalmembranstrukturen ist die Grundlage für zelluläre Adhäsion, Migration und Proliferation und somit für eine Rezellarisierung in vitro. Dezellularisiertes Tränendrüsengewebe stellt daher eine vielversprechende Matrix für die Regeneration von Tränendrüsengewebe in vitro dar.

2. Keratin films for ocular surface reconstructions

Maria Borelli, Stefan Schrader, Gerd Geerling, Augenklinik

Human amniotic membrane (AM) is frequently used as a substrate for ocular surface reconstruction. Its disadvantages (e.g., reduced transparency and biomechanical strength, heterogeneity depending on donor) create the need for standardized alternatives.

Keratin-derived-films (KF) have been indicated as transferable substrate for cell cultivation and tissue engineering; they are engineered by a multi-step procedure including keratin extraction, neutral and alkaline dialysis, drying and a curing process (1). The film characteristics can be varied by changing the protein composition, adding softening agents or varying the curing temperature and duration. Based on these findings, an optimized protocol was developed.

Keratin films swelling and water absorption, as well as tensile strength and light transmission (UV/VIS) were studied; in vitro experiments were conducted to estimate the growth behaviour of corneal epithelial cells on the keratin films and AM in proliferation studies as well as the seeding efficiency and cell detachment behaviour during trypsinization. The film-forming process resulted in transparent films composed of nanoparticulate keratin structures with improved light transmission and biomechanical strength in comparison to AM. Furthermore, cell behaviour on the films was similar to that found on AM (2).

Before moving to in vivo application of KF the impact of different sterilization procedures and their surgical feasibility were investigated. Human hair KFs were prepared and underwent different sterilization procedures (incubation in 70% ethanol, H2O2 plasma sterilization and steam sterilization in PBS). Optical and biomechanical properties, in vitro cell seeding efficiency and proliferation of human corneal epithelial cells on sterilized films were studied and compared with AM. Surgical feasibility was tested in a porcine ex vivo model. Sterilized KF showed higher light transmission and significantly higher E-modulus than AM; cell-seeding efficiency and proliferation rate were not affected by the sterilization procedures. Although KF could be surgically handled, suture placement was more difficult compared to AM. These results show that plasma sterilization seems the best sterilization method for KF without affecting cell biology or optical and biomechanical properties (3).

Although the results so far achieved seem very promising some material modifications might be needed before KF may represent a feasible alternative for ocular surface reconstruction.

REFERENCE

1.Reichl, S. Films based on human hair keratin as substrates for cell culture and tissue engineering. Biomaterials, 2009. 30(36): p. 6854-6866.

2. Reichl S, Borrelli M, and Geerling G. Keratin films for ocular surface reconstruction. Biomaterials, 2011. 32(13): p. 3375-86.

3. Borrelli M, R.S., Feng Y, Schargus M, Schrader S, Geerling G., In vitro characterization and ex vivo surgical evaluation of human hair keratin films in ocular surface reconstruction after sterilization processing. Journal of Material Science. Materials in Medicine, 2013. 24(1): p. 221-230.

AG Norbert Goebels

Autoimmune encephalopathies: Expression of patient derived antineuronal antibodies in recombinant form and analysis of their properties & role in pathogenesis

Manish Malviya, Norbert Goebels
Neurology

The autoimmune encephalopathies are a recently identified group of conditions associated with autoantibodies against neuronal proteins. A pathogenic role of these antibodies is supported by the response of clinical symptoms to immunotherapy and the correlation between antibody titers and neurological outcome. The cognate targets of some of these antibodies have been identified, e.g. subunits of the AMPA receptor, the GABA B receptor, the NMDA receptor, Leucine rich Glioma Inactivated 1 (LGI1), Glutamic acid decarboxylase (GAD_{65}) and others. Affected patients often display a variety of “psychiatric” symptoms such as behavioural changes, anxiety or psychosis followed by seizures, decline of consciousness, aphasia, and abnormal movements.

Previously, it was shown that oligoclonal CSF immunoglobulins are the products of clonally expanded CSF plasma cells/plasma blasts. At the HHU, we have established a recombinant antibody platform, which allows us to reconstruct the antigen specificity of single human B or plasma cells in the form of recombinant human monoclonal antibodies. Using FACS sorting of individual CSF plasma blasts followed by single cell RT-PCR, cloning and recombinant expression of paired immunoglobulin heavy and light chain genes, we successfully rebuilt intrathecal antibody responses of patients with neuroborreliosis and multiple sclerosis.

Currently, we are applying this method to reconstruct intrathecal autoantibody responses from patients with autoimmune encephalopathies: From clonally expanded CSF plasma blasts of a patient with anti-GAD autoantibodies, which are involved in diabetes mellitus, stiff-person syndrome and cerebellar ataxia, we recently derived five recombinant human monoclonal antibodies, two of which show GAD_{65} specific binding to GAD-transfected cell lines and on sections of monkey cerebellum. Likewise, we are now processing CSF plasma blasts from patients with anti-LGI1 and anti-NMDA receptor antibody associated encephalopathies. From a patient with LGI1 encephalitis we collected a total of 135 single CSF plasma blast cells. Eight different clonally expanded plasma cell families were identified and chosen for the production of monoclonal antibodies. From a patient with anti-NMDA receptor autoantibodies, we FACS sorted 116 single CSF plasma blasts for further processing by single cell RT-PCR.

Ultimately, we intend to analyse a battery of recombinant monoclonal antibodies derived from various patients with autoimmune encephalopathies in-vitro and in-vivo to understand the mechanism of their role in disease pathogenesis.

AG Georg Groth

The F_o subcomplex of chloroplast ATP synthase

Daniel Schlieper[1], Christopher Blum[1], Johannes Schwabroh[1], Holger Gohlke[2], Michael Leven[3], Thomas Kurz[3] , Georg Groth[1]
[1]Biochemische Pflanzenphysiologie, Cluster of Excellence on Plant Sciences (CEPLAS),
[2]Pharmazeutische und Medizinische Chemie, Computational Pharmaceutical Chemistry and Molecular Bioinformatics (CPC)
[3]Pharmazeutische Chemie

Chloroplast F_1F_o-ATP synthase uses the energy of a proton motive force across a membrane to produce ATP. The intramembrane subcomplex F_o resembles a turbine in a hydroelectric power plant. The protons flow from the p-side to the n-side of the membrane. First, they flow through subunit *a* to a ring of 14 subunits *c*. This *c*-ring forms the rotor of the turbine. Its rotation provides the mechanical energy for ATP synthesis in subunit F_1. From subunit *c*, the protons are released into a water channel in the hydrophobic region of the membrane. By molecular dynamic calculations based on our crystal structure of spinach chloroplast *c*-ring (PDB code 2W5J), we have mapped the n-side water channel between subunit *a* and the *c*-ring. Additionally, we tested a range of new inhibitors, which are likely to bind at the interface between the water channel and the lipid phase of the membrane. From the results, we discuss a model of the subunit *a* in contact to the *c*-ring.

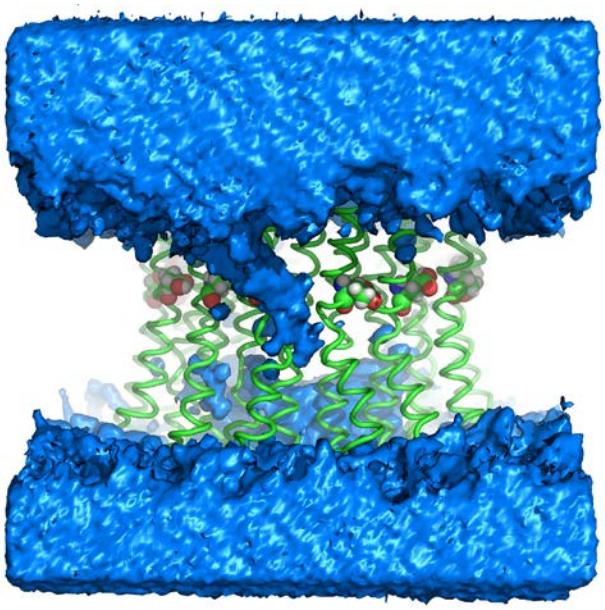

Figure: Molecule dynamics calculations find a water channel from the n-side of the membrane (top) to the proton releasing site of the *c*-ring in the centre. Shown are the *c*-ring (helices) and the water density (solid). The phospholipidic membrane molecules are omitted for clarity. Bottom: p-side of the membrane. (from: Holger Gohlke, Daniel Schlieper & Georg Groth, *J. Biol. Chem.* **287,** 36536-43, 2012).

AG Bernhard Homey

Epidermal EGFR Controls Cutaneous Host Defense and Prevents Inflammation

Beate M. Lichtenberger,1*† Peter A. Gerber,2* Martin Holcmann,1* Bettina A. Buhren,2 Nicole Amberg,1 Viktoria Smolle,1 Holger Schrumpf,2 Edwin Boelke,3 Parinaz Ansari,2 Colin Mackenzie,4 Andreas Wollenberg,5 Andreas Kislat,2 Jens W. Fischer,6 Katharina Röck,6 Jürgen Harder,7 Jens M. Schröder,7 Bernhard Homey,2*‡ Maria Sibilia1*‡
Dermatology

The epidermal growth factor receptor (EGFR) plays an important role in tissue homeostasis and tumor progression. However, cancer patients treatedwith EGFR inhibitors (EGFRIs) frequently develop acneiform skin toxicities,which are a strong predictor of a patient’s treatment response. We show that the early inflammatory infiltrate of the skin rash induced by EGFRI is dominated by dendritic cells,macrophages, granulocytes,mast cells, and T cells. EGFRIs induce the expression of chemokines (CCL2, CCL5, CCL27, and CXCL14) in epidermal keratinocytes and impair the production of antimicrobial peptides and skin barrier proteins. Correspondingly, EGFRI-treated keratinocytes facilitate lymphocyte recruitment but show a considerably reduced cytotoxic activity against Staphylococcus aureus.Mice lacking epidermal EGFR (EGFRDep) show a similar phenotype, which is accompanied by chemokine-driven skin inflammation, hair follicle degeneration, decreased host defense, and deficient skin barrier function, aswell as early lethality. Skin toxicities were not ameliorated in a Rag2-, MyD88-, and CCL2-deficient background or in mice lacking epidermal Langerhans cells. The skin phenotypewas also not rescued in a hairless (hr/hr) background, demonstrating that skin inflammation is not induced by hair follicle degeneration. Treatment withmast cell inhibitors reduced the immigration of T cells, suggesting thatmast cells play a role in the EGFRI-mediated skin pathology. Our findings demonstrate that EGFR signaling in keratinocytes regulates key factors involved in skin inflammation, barrier function, and innate host defense, providing insights into the mechanisms underlying EGFRI-induced skin pathologies.

AG Carsten Korth

1. The tgDimer mouse – a novel model for early events of Alzheimer´s disease

Andreas Müller-Schiffmann[1], Arne Herring[2], Laila Saber[3], Sandra Schäble[3], Heinrich Sticht[4], Kathy Keyvani[2], Maria A. de Souza Silva[3], Joseph P. Huston[3] , Carsten Korth[1]
[1)] Institute for Neuropathology, [2)] Institute of Pathology and Neuropathology, University Hospital Essen, [3)] Center for Behavioral Neuroscience, [4)] Institute for Biochemistry, Friedrich-Alexander-University Erlangen-Nürnberg

Recent evidence indicates a pivotal role for Abeta dimers in the early course of Alzheimer´s disease. However, analysis of dimers is hampered by low availability and a weak conformational stability. Thus, it remains difficult to dissect the effects of *in vivo* generated soluble dimers from simultaneously present assemblies of higher structured oligomers, protofibrils and fibrils. We have recently shown that exchange of a serine residue to a cysteine in position 8 of Abeta leads to efficient and stable production of elongation-incompetent, disulfide stabilized Abeta-dimers in a cell culture system. Abeta-S8C dimers that were purified by size exclusion chromatography displayed a high synaptotoxicity in picomolar concentrations as revealed by whole cell patch clamp analysis.

To characterize the functions of these Abeta dimers *in vivo* we established a tg-APP-Abeta-S8C mouse line (tgDimer). The Abeta-S8C mutation was introduced into pTSC21-APP751swe allowing the Thy1.2 promoter-driven neuron-specific expression of APPswe-Abeta-S8C. Three lines with different expression levels of APP-Abeta-S8C were established and analysed by means of biochemistry and histopathology.

APP-Abeta-S8C expression exceeds that of endogenous APP by a factor of approximately 10-fold on the protein level. In brain homogenates, exclusively dimeric Abeta-S8C, but not monomeric or higher structured Abeta was detected. These Abeta dimers stay soluble over time, since no insoluble Abeta can be extracted from brains of 24 months old tgDimer mice. This is in accordance to a complete absence of amyloid plaques after histologicial analysis of the same mice. Likewise, we detected no signs of astrogliosis and microgliosis in aged tgDimer mice. Thus, late symptoms of Alzheimer´s disease pathology are lacking in the tgDimer model. However, early events like tau-hyperphosphorylation are present even in young tgDimer mice (6 months) and thus most likely are mediated by dimeric Abeta rather than fibrillar Abeta. We have also performed behavioral studies that will be reported.

The tgDimer mouse line allows the specific identification of functions exclusively mediated by Abeta dimers in the absence of Abeta plaques and may serve as a valuble model for the early stages of Alzheimer´s disease. Moreover it provides a basis for testing of novel treatment strategies targeting Abeta-dimer mediated synaptotoxicity.

2. Dopamine regulation by functional DISC1 aggregates

Verian Bader1+, Svenja V. Trossbach1+, Martin Pum2, Ingrid Prikulis1, M. Angelica de Souza Silva[2], Phebian A. Babalola[3], Zoë A. Hughes[3], Susan F. Godsave[4], Peter J. Peters[4], Gary W. Miller[5], Amy Ramsey[6], Nicholas J. Brandon[6,7], Joseph P. Huston[2], Carsten Korth[1] *
[1] Department Neuropathology, Heinrich Heine University of Düsseldorf, Germany, [2] Center for Behavioral Neuroscience, Heinrich Heine University of Düsseldorf, Germany, [3] Department of Psychiatry and Behavioral Disorders, Pfizer Neuroscience, Cambridge, MA, USA, [4] Netherlands Cancer Institute, Amsterdam, The Netherlands, [5]Department of Environmental Health, Rollins School of Public Health, Emory University, Atlanta, GA, USA
[6]Department of Pharmacology and Toxicology, University of Toronto, Toronto, Canada, [7]Current address: AstraZeneca Neuroscience iMED, Cambridge, MA, USA
+ these authors contributed equally

Background
A characteristic hallmark feature of neurodegenerative diseases like Alzheimer's and Parkinson's disease is a disturbed cellular proteostasis reflected by insoluble protein and intracellular protein aggregates like Lewy bodies. Schizophrenia is a chronic, multifactorial psychiatric disease that is characterized by dysfunctional dopamine metabolism involving the medial prefrontal cortex and the striatum. In a recent study we demonstrated insoluble DISC1 protein in a subgroup of patients with chronic mental disease. Here, we show that DISC1 protein solubility is related to dopamine metabolism.

Methods
To address the interaction of DISC1 protein and dopamine metabolism in vitro, we generated a doxycycline-inducible neuroblastoma cell line expressing non-mutant DISC1. We performed detailed biochemical and immunocytochemical analysis and developed assays to quantify dopamine metabolism properties by reporter assays and HPLC analysis. To model human DISC1 aggregation and its influence on dopamine metabolism in vivo, we generated a transgenic rat model expressing non-mutant human DISC1.

Results
In response to high extracellular DA concentrations, DISC1 displays distinct perinuclear aggresomes that are reversible after treatment washout. In addition a high molecular weight band in Western Blot is formed that is paralleled by suppressed clearance of extracellular DA. These effects were mediated by a functional interaction with the Dopamine transporter (DAT). Transgenic rats with detectable DISC1 aggregates proved to be hypersensitive to amphetamine indicating a DA imbalance.

Discussion
We suggest a biological function for DISC1 aggregates in the regulation of extracellular DA levels mediated by a functional interaction with DA transporter systems, in particular the Dopamine transporter. DISC1 aggregates as cytosolic sensor for DA load might harbor an important function in regulating the DA-quinone mediated oxidative stress by modulation the uptake of extracellular DA. The proposed mechanism might be important for the understanding of mental illnesses that are driven by dopamine imbalances.

Funding: DFG KO1679/3-1, NEURON-ERANET DISCover (BMBF 01EW1003)

AG Karl Köhrer

Next Generation DNA Sequencing: Strategies for targeted resequencing

Stefanie Stepanow, René Deenen, Karl Köhrer
Genomics and Transcriptomics Laboratory (GTL), BMFZ

Work in the Genomics and Transcriptomics Laboratory (GTL) of the BMFZ concentrates on the analysis of DNA and RNA. Capillary sequencing and DNA microarray technologies are routinely used for the analysis of single or multiple samples in parallel. Next Generation DNA sequencing technologies are used for the analysis of multiple and/or complex heterogeneous samples, addressing whole genomes or subgenomic regions.

In the first part of the talk, we will present protocols and results for targeted resequencing projects addressing disease-related subgenomic regions and whole exomes. We will show validation and optimization of variant and insertion / deletion detection results.

The second section will address resequencing of variable regions in bacterial 16S rRNA genes to get insights into the composition of complex microbial communities.

We will present and discuss various sample preparation options, present Ion sequencing data, and also downstream analysis options.

Contact:
Stefanie Stepanow – Tel. 81 10475 – Email Stefanie.Stepanow@uni-duesseldorf.de
René Deenen – Tel. 81 10475 – Email Rene.Deenen@uni-duesseldorf.de
Karl Köhrer – Tel. 81 13165 – Email koehrer@uni-duesseldorf.de

AG William Martin

Early infection dynamics of Trichomonas vaginalis

Gary Kusdian, Christian Wöhle, Verena Zimorski, William F. Martin, Sven B. Gould
Molekulare Evolution

Trichomonas vaginalis is the causative agent of the most common sexually transmitted disease trichomoniasis. The protist is a free-swimming flagellated cell and undergoes a dramatic morphologic change, when in contact with tissue of the human urogenital tract. It turns into an amoeboid-adherent cell migrating on host cells, which is an essential mechanism for the parasite to successfully infect. The molecular machinery behind this transition is not well studied. To identify potential key players we used comparative transcriptomics on a mixture of parasite and host mRNA and downstream bioinformatic screening. Among the candidates we identified a protein of the ancient eukaryotic fimbrin family, which inhabits two actin-binding domains. *Tv*FIM1 increases the speed of actin polymerization and bundles F-actin in a parallel and anti-parallel manner. During infection the protein experiences a dramatic re-localization and associates with structures reminiscent of actin cables and at protruding sites of pseudopodia, suggesting the protein to be involved in the parasite‘s highly dynamic amoeboid movement across host tissue. Our transcriptomic data furthermore provides insight into the action-response pattern of parasite and host during the infection process.

AG Hans Werner Müller

Identification and Characterization of regeneration-associated genes (RAGs) by paradigm-specific gene expression profiling of injured PNS

Frank Bosse, Hans Werner Müller
Molecular Neurobiology Laboratory, Department of Neurology

In order to identify regeneration-associated genes we performed a comprehensive series of mRNA expression studies at 8hrs, 1d, 2d, 7d and 21d after rat sciatic nerve injury. Expression profiles of three distinct lesion paradigms of the sciatic nerve were obtained by Affymetrix GeneChip hybridization: (i) during Wallerian degeneration and spontaneous regeneration after peripheral nerve crush, (ii) of chronically axotomized neurons after nerve transection combined with ligation of nerve stumps, and (iii) during delayed regeneration of neurons following reanastomosis (coaptation) of previously cut nerve stumps.

Clustering of lesion-induced mRNA regulations enabled us not only to determine distinct *paradigm*- as well as *time point-specific* expression patterns but also to identify numerous new putative regeneration-associated genes (RAGs) that exactly reflect expression patterns of well established genes with crucial functions in successful axonal regeneration.

In order to characterize selected new putative RAGs we started cellular localization analysis *in vivo* as well as functional tests *in vitro*. Interestingly, cultures of neuronal F11 cells showed differential mRNA expression of a large proportion of the selected putative RAGs under neurite outgrowth promoting culture conditions. We successfully characterized the cellular distribution of such selected putative new RAGs and revealed expression in large and small diameter sensory DRG neurons *in vivo.*

To assess whether these RAG-candidates may affect neurite outgrowth, specific constructs were generated to recombinantly modify candidate gene expression levels in cultured F11 cells under different growth/differentiation conditions. Functionality of generated constructs were validated by quantitative RT-PCR as well as western blots. In fact, transient overexpression and/or suppression studies revealed distinct impact of several putative RAG-candidates on both neurite formation and neurite outgrowth. In particular, transient overexpression of specific RAG-candidates was sufficient to induce the generation of neurite-like processes under proliferating culture condition and to promote neurite outgrowth. Additional co-transfection experiments with combinations of RAG-candidate genes suggest additive effects on neurite outgrowth. Expectedly, transient knock-down of RAG-candidates using gene-specific siRNA-Pools resulted in a decrease of both total neurite number and neurite length in the majority of cases suggesting functional role(s) of these genes in the context of axonal regeneration.

Further investigations are started to elucidate the functional roles und participating signaling pathways of the new RAG candidates in the context of neurite outgrowth in more detail.

Supported by BMFZ and the JÜRGEN MANCHOT STIFTUNG

AG Klaus Pfeffer

The lymphotoxin-β receptor (LTβR) and its role in hepatocyte-mediated liver regeneration

Kristina Behnke, Ursula Sorg, Klaus Pfeffer
Medical Microbiology and Hospital Hygiene

The liver retains a capacity for regeneration in response to injury. Loss of at least 30 % of liver mass leads to synchronized proliferation of mature hepatocytes (compensatory hyperplasia). During regeneration, the liver continues to perform its essential functions, such as protein synthesis, glycogen storage, bile secretion, and coagulation. It has previously been shown that mice deficient in LTβR ($LT\beta R^{-/-}$) exhibit reduced survival after partial (70 %) hepatectomy (PHx).

Our data confirms that $LT\beta R^{-/-}$ mice have a decreased survival rate compared to WT mice 62 % vs. 90 %, respectively). Liver sections of $LT\beta R^{-/-}$ animals showed a higher number of necrotic areas and more vacuolisation of hepatocytes 24 h and 48 h post PHx. Microarray analysis and quantitative RealTime PCR identified a panel of differentially expressed genes, including cell cycle regulators, cytokines and serum proteins. In addition, the cytokine expression profile of $LT\beta R^{-/-}$ animals is altered and several serum proteins appear to be deregulated (e.g. a significant increase in alkaline phosphatase in $LT\beta R^{-/-}$ animals 24 and 48 h post PHx). Interestingly bile acids, which are known to play a key role in initiating liver regeneration were elevated in $LT\beta R^{-/-}$ animals.

Our findings demonstrate that a deficiency in LTβR signalling leads to substantial changes in liver morphology, gene expression, cytokine levels and other serum parameters. This clearly demonstrates the importance of LTβR signaling in liver regeneration and its exact mechanism will be elucidated in further studies.

AG Guido Reifenberger

Establishment of an automated high-throughput functional screening of the microRNAome in glioma cells

Bastian Malzkorn, Tanja Wader, Guido Reifenberger,
Neuropathology
E-mail: bastian.malzkorn@uni-duesseldorf.de

MicroRNAs (miRNAs) are approximately 22 nucleotides long, non-protein-coding RNA molecules that post-transcriptionally regulate gene expression and that were shown to influence glioma cell proliferation and apoptosis. A single miRNA can regulate multiple target mRNAs and a single target site can be regulated by a family of miRNAs that share the same seed sequence. Up-regulation of a certain miRNA may be compensated by down-regulation of a functionally homologous miRNA family member. These findings suggest that - on the basis of miRNA expression data - the functional relevance of miRNAs in gliomas is difficult to predict. Therefore, we established a new approach for investigating the role of miRNAs in glioblastoma pathogenesis using the Beckman Coulter Biomek FXP workstation for an automated high-throughput miRNA inhibitor-based functional screening that covered the whole miRNAome. We implemented standard operating procedures for the cultivation of glioma cell lines (U-87MG, LN-229, T98G, U-138MG) that included authentification, mycoplasma testing, standardized subcultivation routines, systematic expansion, and cryopreservation of glioma cell lines. In addition, we optimized the conditions of transient transfection of glioma cells with miRNA inhibitors in order to achieve high transfection efficiency and efficacy combined with low cytotoxicity. To compare the effects of different miRNA inhibitors on glioma cell growth we applied a commercially available luminescent growth assay, the results of which correlated with cell number across a big range of values. Furthermore, we established algorithms for the automation of all experimental steps of the functional screening in order to create highly similar experimental schedules as well as homogenous results within an assay plate and between different plates and different batches. After comparing several methods of data transformation, normalization and aggregation we developed an algorithm of comprehensive data analyses using open-source statistical packages. In summary, we established a pipeline of experimental procedures and data analyses for an automated high-throughput functional screening of the miRNAome in order to reliably identify novel miRNAs that promote glioma cell growth.

Acknowledgement: This study is supported by a start-up grant from the “Forschungskommission der Medizinischen Fakultät der Heinrich-Heine-Universität Düsseldorf” (grant no. 45/2010)

AG Brigitte Royer-Pokora

Systembiologie des erblich bedingten Kolonkarzinoms (HNPCC)

K. Hardt, Nils Rahner, Beate Betz, Brigitte Royer-Pokora
Humangenetik und Anthroplogie

Ziel von HNPCC-Sys ist die Entwicklung neuer Diagnosestrategien zur Vorhersage des Tumorrisikos und Prävention basierend auf einem systembiologischen Ansatz.

Im Rahmen von HNPCC-Sys sollen von 360 Patienten aus dem Ruhr-Rhein-Gebiet bei ihrer jährlichen Vorsorge-Darmspiegelung Gewebeproben aus dem Darm entnommen werden. Im Fall eines Adenoms oder Karzinoms werden die Gewebe am MPI in Berlin genomweit sequenziert. Die dadurch erhobenen Daten werden in Düsseldorf auf relevante Mutationen analysiert, validiert und funktionell getestet.

In einer ersten Analyse werden die ausgewählten Gene auf ihre funktionelle Vernetzung in bekannten Pathways hin analysiert. Dafür sollen verschiedene öffentlich zur Verfügung stehen Programme, wie z.B. DAVID, und lizenzpflichtige Programme, wie z.B. GeneGo, eingesetzt werden. Ergibt sich eine interessante Vernetzung mit tumorrelevanten Pathways sollen diese Gene als erstes weiter untersucht und biologisch charakterisiert werden.

Die entsprechenden Gene werden als Expressionskonstrukte fertig bestellt oder selbst hergestellt und die ausgewählten Mutationen eingefügt. Für die knock-down Analysen werden die sh-Lentiviren hergestellt oder kommerziell bezogen. In der Humangenetik in Düsseldorf stehen bereits eine Reihe von Kolonkarzinomzelllinien und lentiviralen Vektoren zur Verfügung, die für Überexpressions- und ‚knock down' Experimente verwendet werden können**.**

In Kolonkarzinomen ist häufig der Wnt- sowie der TGFß- Signalweg dereguliert. Deshalb werden wir untersuchen, ob eine mögliche Interaktion der gefundenen mutierten Gene mit diesen Signalwegen besteht. Die interessanten ausgewählten Gene werden auf ihre subzelluläre Lokalisation in Tumorzellen analysiert, sowie in knock-down und Überexpressionsanalysen weiter charakterisiert. Das Differenzierungspotential von Kolonkarzinomzelllinien wird nach knock-down von putativen Onkogenen oder Überexpression von putativen Tumorsupressorgenen untersucht.

Die biologischen Eigenschaften der entsprechend manipulierten Zellen wird in Invasions- und Migrationsanalysen getestet, sowie die Apoptoseinduktion untersucht. Letztendlich soll auch das Genexpressionsmuster nach entsprechender Manipulation der Zellen bestimmt werden, um weitere Einblicke in deren Funktion zu erhalten.

AG Heiner Schaal

HEXploring of the HIV-1 genome allows landscaping of new potential splicing regulatory elements

Steffen Erkelenz[1,*], Stephan Theiss[1,*], Marianne Otte[2], Marek Widera[1], Jan Otto Peter[1], Heiner Schaal[1]
[1]Virology, [2]Genetics, [*]These authors contributed equally to this work.

Effective selection between true and decoy splice sites is critically controlled by flanking splicing regulatory elements (SREs), which can enhance or repress splice site use. Recent experimental evidence suggests that the entire regional context of SREs rather than a single enhancer/silencer hexamer jointly contribute to splicing.

Extending the hexamer score concept [Fairbrother et al. (2002) Science 297:1007-13], we represent the splicing regulatory property of an entire 5'ss neighborhood by a weighted average of normalized Z-scores for all hexamers overlapping with the target region. These novel "HEXplorer" scores describe the degrees of exon- or intron-likeness (Z_{EI}) and enhancer-likeness (Z_{WS}) for a given region upstream of a 5'ss. These scores can be graphically represented by positive or negative oriented areas along the sequence. Mutation effects on an entire 5'ss neighborhood are then captured by comparing the HEXplorer areas of wild type and mutant sequences upstream of a 5'ss. The fundamental datasets of weak and strong 5'ss used in the definition of these HEXplorer scores were derived based on the HBond score that measures the 5'ss complementarity to U1 snRNA.

In a first test, we scanned the small *non*-coding HIV-1 leader exon 3 for regions enriched in SREs. Here, HEXplorer scores correctly indicated both the well-known exonic splicing silencer ESSV and the recently discovered exonic splicing enhancer ESE_{vpr} upstream of 5'ss D3.

Next, we tested the HEXplorer's capability to predict mutations' potency to modify 5'ss D3 usage. We systematically examined this ESE region using various single and double mutations predicted to either alter 5'ss usage or act neutrally. In 20 tested mutations, the HEXplorer prediction correlated well with the experimentally detected level of exon inclusion.

Extending the HEXplorer approach to all HIV-1 exons, we were able to identify three novel exonic splicing enhancers that contribute to the inclusion of the viral exons 2, 2b and exon 4. All three novel ESEs were experimentally confirmed by HEXplorer predicted point-mutations. Beyond application to HIV-1 5'ss usage, the HEXplorer may also prove particularly useful as a method for assessing pathogenic human exonic mutations.

AG Jürgen Scheller

Bacterial expression of p35 as a novel strategy to dissect IL-12 and IL-35 functions

Jens Moll, Samadhi Aparicio-Siegmund, Svenja Plöhn, Christoph Garbers, Jürgen Scheller
Biochemistry and Molecular Biology II

The pro-inflammatory cytokine IL-12 is secreted by antigen-presenting cells including macrophages and dendritic cells. IL-12 is crucial for adaptive cellular immunity and has immunomodulatory and anti-angiogenic functions. The heterodimeric cytokine consists of two disulfide-linked subunits, p40 and p35, and forms a signaling complex with the membrane-bound ß-receptors IL-12Rβ1 and IL-12Rβ2, which activates intracellular signaling pathways like Jak/STAT. Interestingly, the p40 subunit has been described to be essential for p35 stabilization and subsequent IL-12 secretion, as p35 alone is not secreted from cells. Consequently, dissection of individual functions of p35 in the absence of the p40 subunit is a challenging task.

We developed a strategy to express, purify and refold murine p35 in E.coli. The p35 subunit did not induce activation of the Jak/STAT pathway when combined with p40. A single point mutation in p35 led, however, to the formation of biologically active IL-12 (p35/p40 heterodimers) as judged by proliferation of Ba/F3-IL-12Rβ1/IL-12Rβ2 cells and phosphorylation of STAT1 and STAT3. It is tempting to speculate that our biologically active p35 will allow the in vitro formation of the heterodimeric cytokine IL-35 (p35/EBI3). This may provide an avenue for functional studies of IL-35 independently of IL-12 effects. Our findings will provide means to further unravel the complex functional network of cytokine cross-talk within the IL-6 superfamily and address the individual biological functions of IL-12 and IL-35.

AG Wolfgang Schulz

Expression and epigenetic regulation of LINE-1 retroelements in human urothelial cancer

Ulrike Kreimer, Wolfgang Goering, Günter Niegisch, Wolfgang A. Schulz
Urology

Background: Genome-wide hypomethylation during bladder tumorigenesis is thought to lead to decreased methylation of LINE-1 sequences, the most frequent family of retroelements in the human genome, to enable LINE-1 transcription and thereby contribute to chromosomal instability. We therefore assessed individual and global LINE-1 methylation and expression in bladder cancer tissues and cell lines.

Material and methods: Global full-length LINE-1 methylation and expression were determined in 11 benign and 24 bladder cancer tissues, six primary urothelial cell cultures and 18 bladder cancer cell lines. Eight individual LINE-1 loci were assessed for expression in the same sample set. Methylation of 11 single LINE-1 loci were analysed in paired benign and tumor samples of 16 bladder tissues by pyrosequencing. Results were compared to clinical parameter. **Results**: Globally, LINE-1 methylation decreased significantly in bladder cancer tissues and cell lines, which was associated with an overall increase in LINE-1 mRNA, especially in full-length transcripts. At single LINE-1 loci DNA methylation changes occurred in an essentially stochastic fashion during urothelial cancer development. LINE-1 transcripts from three loci were detected in tissues samples but quantification failed due to low expression values. Overall as well as individual LINE-1 methylation and expression do not correlate with any clinical parameter. **Conclusion**: Global LINE-1 hypomethylation is associated with significant activation of overall LINE-1 expression in bladder cancers but these changes were not caused by the analysed subset of individual LINE-1s. To determine the LINE-1 loci causative for the observed global changes high-throughput sequencing of a larger number of full-length LINE-1s will be necessary. Supported by the Reinhard-Nagel-Stiftung

AG Holger Schwender

Bioinformatic and biostatistical consulting and services for the BMFZ

Rafael Dellen, Razif Gabdoulline, Wolfgang Kaisers, Holger Schwender
Center of Bioinformatics and Biostatistics (CBiBs), BMFZ

The Center of Bioinformatics and Biostatistics (CBiBs) at the BMFZ provides bioinformatic and biostatistical consulting and services in particular for the researchers at the BMFZ, but also for all other members of the Heinrich Heine University and the Düsseldorf University Hospital. Moreover, we provide short courses for statistical and bioinformatic software.

In our talk, we will give a brief overview on the consulting and the courses provided by the CBiBs since the last BMFZ retreat. As an example for our bioinformatics services, we will present the bioinformatic results of a joint project with the Department of Neuropathology and the Molecular Proteomics Laboratory concerned with the detection of genetic variants contributing to the development of temozolomide (TMZ) resistance in glioblastoma.

Exome-wide sequencing of TMZ sensitive and TMZ resistant cell lines led to the identification of a few thousands variants in at least one of these cell lines. To reduce this large number of variations, we developed a tool that combines database and quality information on the variants with other genomic data (here, gene expression and aCGH data), and thus, allows selecting the most promising candidates for a validation studies. To additionally consider proteomic information and compare the results of the exome sequencing analysis with the ones of the proteomic analysis, we also developed a software enabling the comparison of the trypsinated mutated peptide sequences resulting, on the one hand, from the mutated genomic sequences, and on the other hand, from the proteomic experiments.

These two and other softwares/pipelines can be easily adapted to other, related genomic and/or proteomic problems and are available for all members of the BMFZ.

AG Kai Stühler

Quantitative protein mass spectrometry

Kai Stühler, Gereon Poschmann, Daniel Waldera, Anja Stefanski
Molecular Proteomics Laboratory, BMFZ

Protein mass spectrometry (MS) has rapidly developed in the last years and beside other applications in proteomics it is meanwhile broadly applied for a wide range of protein analytical questions like for instance characterization of proteins' primary, secondary and tertiary structure, identification of posttranslational modifications and even absolute protein quantification.

At the Molecular Proteomics Laboratory (MPL) electrospray ionization (ESI) MS instruments coupled with high performance nano liquid chromatography (nano LC-ESI-MS) systems have been establish to address particularly quantitative question in biological research. The Orbitrap Elite® system is commonly used for high resolution measurements which are necessary for discovery driven analysis of complex protein mixtures using e.g. SILAC or label-free MS. For targeted quantification of proteins the TSQ Vantage® system is applied at the MPL. This triple-quadrupol MS instrument allows the relative and absolute quantification of known proteins by selected reaction monitoring (SRM)-analysis. Here, we will present actual application of protein mass spectrometry in collaboration with groups from the Biologisch-Medizinisches-Forschungszentrum.

AG Markus Uhrberg

Phenotypic and functional NK cell deficiencies in patients with Myelodysplastic Syndrome

Hejazi M, Manser A, Fröbel J, Kündgen A, Haas R, Germing U, Gattermann N, Uhrberg M,
Transplantation Diagnostics and Cell Therapeutics
Hematology, Oncology and Clinical Immunology

Myelodysplastic syndrome (MDS) is a heterogeneous clonal disorder characterized by ineffective hematopoiesis that can progress to acute myeloid leukemia (AML). The role of NK cells in etiology and immune surveillance of MDS is unclear. Here, based on phenotypic and functional analysis of NK cells, newly diagnosed MDS patients (n = 60) could be subdivided into three groups with I. normal NK cell counts and function; II. normal or reduced NK cell counts but impaired NK cell function; III. a general lack of NK cell (< 10 cells/µl peripheral blood). In patients with impaired NK cell function (group II) IFN-ɣ production and cytotoxicity against K562 target cells was strongly reduced. Surprisingly, degranulation of MDS-NK cells in response to target cells (CD107 assay) was normal when compared to NK cells of healthy donors. However, the degranulated NK cells contained low intracellular levels of granzyme B and perforin explaining the impaired cytotoxicity of NK cells in group II patients. The general lack of NK cells seen in group III patients was selective and not associated with a reduction of T cells. To assess if this NK deficiency was due to an intrinsic defect of hematopoietic progenitor cells (HPC), CD34+ HPC were isolated from bone marrow of MDS patients and subjected to an in vitro NK cell differentiation assay. Indeed, phenotypically mature and functional NK cells could be generated from group III patients, albeit at a decreased frequency compared to HPC isolated from bone marrow of healthy age-matched controls.

The present analysis reveals widespread defects in the NK cell compartment of MDS patients and suggests that a non-functional bone marrow niche contributes to the unusual lack of NK cells seen in a subset of MDS patients. As reduced frequencies of NK-cells correlated with poor prognosis (IPSS score), analysis of NK cells could provide a novel prognostic parameter in MDS.

AG Sascha Weggen

Generation of a faithful model for familial Alzheimer's disease with presenilin-1 mutations by dual recombinase-mediated cassette exchange

Vanessa Kurth, Sascha Weggen
Neuropathology

Background: The vast majority of familial Alzheimer's disease (FAD) cases with autosomal-dominant inheritance harbor heterozygous mutations in the presenilin-1 (PSEN1) gene. PSEN1 mutations increase the proportion of the aggregation-prone Abeta42 peptide species. In addition, PSEN1 mutations have been proposed to impair crucial cellular processes including signal transduction and calcium homeostasis. However, these effects and their potential contribution to the clinical phenotype of FAD remain controversial, likely due to the use of overexpression models that did not accurately reflect the genetic background and the biochemical composition of the gamma-secretase complex in FAD patients.

Objectives: To incorporate specific mutations into the endogenous PSEN1 gene in mouse embryonic stem (ES) cells by an innovative gene-targeting strategy termed dual recombinase-mediated cassette exchange (dRMCE).

Methods: dRMCE takes advantage of pre-targeted mouse alleles such as those generated by the International Knockout Mouse Consortium (IKMC). In these conditional alleles, the target sites for Cre and Flp recombinases can be exploited to re-engineer the genomic locus with high frequency.

Results: To introduce mutations into the conditional PSEN1 allele, we have generated replacement constructs encompassing exons 5-12 corresponding to 75% of the mouse PSEN1 protein flanked by FRT and loxP recombination sites. These constructs were co-transfected with a vector encoding both Flpo and iCre into ES cells containing the PSEN1 conditional allele. For constructs encoding either wild type or mutant PSEN1, extensive validation by PCR demonstrated successful replacement of the conditional allele with a frequency of approximately 30%. To verify equal expression of both the re-engineered PSEN1 allele and the second unmodified allele, we compared the levels of PSEN1 protein in cell clones that had undergone successful recombination with negative cell clones that contained only one functional PSEN1 allele. Densitometry analysis demonstrated that the PSEN1 expression was twice as high in positive as compared to negative cell clones, indicating that the replaced locus was fully functional and expressed normal levels of a correctly spliced PSEN1 mRNA.

Conclusions: The lack of consensus concerning the effects of PSEN mutations indicates the need for improved cellular models that account for the heterozygous expression of PSEN mutants. Our approach based on ES cells and genome editing is a cost-effective alternative to primary or iPS cells, and provides an FAD model suitable for stringently controlled biochemical and kinetic experiments. Moreover, dRMCE is applicable to the study of genetic mutations in almost any protein at native levels.

AG Sebastian Wesselborg

Role of the Ulk1-Atg13-FIP200-Atg101 Complex in the Regulation of Autophagy: Crosstalk, Shortcuts, and Feedbacks

Björn Stork, Sebastian Wesselborg
Molekulare Medizin

(Macro-)Autophagy is an evolutionary extremely conserved process, and the morphologic identification of autophagy was reported in the 1960s. Autophagy is a degradative process in which cytoplasmic components are sequestered by double-membraned vesicles called autophagosomes and subsequently delivered to lysosomes. Generally, autophagy is induced when cells are deprived of nutrients in order to maintain the amino acid pool. However, different alternative physiological roles have been proposed for autophagy, i.e. intracellular quality control, cell death, or tumour suppression. The Ulk1-Atg13-FIP200-Atg101 complex is a central mediator of autophagic processes. If nutrients are sufficiently available, the mammalian/ mechanistic target of rapamycin complex 1 (mTORC1) associates with the Ulk1-Atg13-FIP200-Atg101 complex, leading to the phosphorylation of Ulk1 and Atg13, and to the inhibition of autophagy. In contrast, rapamycin treatment or starvation lead to the dissociation of mTORC1 from the Ulk1-Atg13-FIP200-Atg101 complex, resulting in a dephosphorylated status of Ulk1 and Atg13. This in turn leads to Ulk1 autophosphorylation and Ulk1-mediated phosphorylation of Atg13 and FIP200. These phosphorylations ultimately trigger the induction of autophagy. Here, an overview about our recent work on the Ulk1-Atg13-FIP200-Atg101 complex will be presented, including the identification of an Ulk1-dependent negative feedback signaling circuit and the characterization of Ulk1-independent induction of autophagy.

AG Dieter Willbold

1. Quantitation of single protein aggregates for diagnostics of protein misfolding diseases

Oliver Bannach, Detlev Riesner, Eva Birkmann, Dieter Willbold
Physikalische Biologie

The prototype of protein misfolding diseases is the transmissible spongiform encephalopathy, or prion disease. According to the prion hypothesis a misfolded conformer of the ubiquitous prion protein, designated PrP^{Sc}, is the causative agent of the disease. Unique biochemical features of PrP^{Sc} like its aggregated state can be exploited as biomarker to diagnose infected individuals.

Previously we described surface-based fluorescence intensity distribution analysis (sFIDA) as diagnostic tool for prion disorders, such as Scrapie and BSE (Bannach et al., 2012; Bannach et al., 2013; Birkmann et al., 2007). In sFIDA, PrP aggregates are fixed on a capture-coated glass surface and loaded with at least two antibodies which carry different fluorescence dyes. Dual-color measurements allow discrimination of cellular PrP and disease-associated PrP aggregates, because only multimeric PrP can be both captured and probe-labeled, respectively. Single PrP particles on the chip surface can be visualized by standard fluorescent microscopy techniques, including confocal laser scanning and total internal reflection fluorescence microscopy. We established sFIDA as highly sensitive diagnostic tool using brain tissue, CSF, and even blood samples. We could successfully discern healthy and infected animals like hamster, cattle, and sheep.

The sFIDA assay is a universal tool for detection of aggregated proteins. Recently we adapted the method for diagnostics of other protein misfolding diseases including *Morbus Parkinson* and *Morbus Alzheimer* (Hubinger et al., 2012; Wang-Dietrich et al., 2013). By sFIDA analysis of spinal fluid, a cohort of AD-affected patients could be distinguished significantly from an age-matched control group.

Bannach, O., Birkmann, E., Reinartz, E., Jaeger, K.E., Langeveld, J.P., Rohwer, R.G., Gregori, L., Terry, L.A., Willbold, D., Riesner, D., 2012. Detection of prion protein particles in blood plasma of scrapie infected sheep. PloS one 7, e36620.

Bannach, O., Reinartz, E., Henke, F., Dressen, F., Oelschlegel, A., Kaatz, M., Groschup, M.H., Willbold, D., Riesner, D., Birkmann, E., 2013. Analysis of prion protein aggregates in blood and brain from pre-clinical and clinical BSE cases. Veterinary microbiology 166, 102-108.

Birkmann, E., Henke, F., Weinmann, N., Dumpitak, C., Groschup, M., Funke, A., Willbold, D., Riesner, D., 2007. Counting of single prion particles bound to a capture-antibody surface (surface-FIDA). Veterinary microbiology 123, 294-304.

Hubinger, S., Bannach, O., Funke, S.A., Willbold, D., Birkmann, E., 2012. Detection of alpha-synuclein aggregates by fluorescence microscopy. Rejuvenation research 15, 213-216.

Wang-Dietrich, L., Funke, S.A., Kuhbach, K., Wang, K., Besmehn, A., Willbold, S., Cinar, Y., Bannach, O., Birkmann, E., Willbold, D., 2013. The amyloid-beta oligomer count in cerebrospinal fluid is a biomarker for Alzheimer's disease. Journal of Alzheimer's disease : JAD 34, 985-994.

2. Non-conventional approaches for structural and functional studies of membrane proteins

Manuel Etzkorn, Dieter Willbold
Physikalische Biologie

A new independent research group at the institute of Physical Biology is introduced. Funded by the Emmy-Noether Program of the DFG we focus on the development of novel NMR methodology with an emphasis on its applicability in demanding biological systems. Investigation of membrane protein folding, structure and function were selected to offer a well suited (i.e. challenging) target for the application of the new techniques. The presentation will give a short overview on several non-conventional methods that we will establish/use including cell-free protein expression, amphipathic polymers, and lipid bilayer nanodiscs as well as interleaved NMR spectroscopy. In addition the selected protein systems will be briefly introduced.

3. Anfahrtsbeschreibung

Maria in der Aue
Tagungsstätte des Erzbistums Köln
In der Aue 1
42929 Wermelskirchen

Fon (02193) 505-0
Fax (02193) 505-101

E-mail: empfang@tagungen-aue.de
www.tagungen-aue.de

Mit öffentlichen Verkehrsmitteln:
Bis Köln Hbf. Weiter mit der S-Bahn (S 11) bis Berg.- Gladbach. Anschließend mit dem Taxi ca. 15 km Richtung Wermelskirchen-Dabringhausen fahren.

Mit dem PKW:
Über A1: Abfahrt Nr. 97 Burscheid, links Richtung Wermelskirchen-Hilgen. 2. Ampel rechts Richtung Dabringhausen durch den Wald bis zur Kreuzung. Rechts Richtung Altenberg, nach ca. 1,2 km in Limmringhausen / Bremen links abbiegen. Ca. 2 km bis zum Haus fahren. Ausreichend kostenfreie Parkplätze vor Ort.

Detailliertere Anfahrtsbeschreibung unter:
http://tagen.erzbistum-koeln.de/aue/kontakt/anfahrt/
