Abstracts

XXV. Jahrestagung der Kind-Philipp-Stiftung für Leukämieforschung in Wilsede vom 6. – 9. Juni 2012

Ausrichter (organized by):
Zentrum für Geburtshilfe, Kinder- und Jugendmedizin, Klinik für pädiatrische Hämatologie und Onkologie, Universitätsklinikum Hamburg-Eppendorf; Klinik für Allgemeine Pädiatrie, Universitätsklinikum Schleswig-Holstein, Campus Kiel; Institut für Pharmazeutische Biologie, Goethe Universität Frankfurt am Main

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- **PEQLAB Biotechnologie GmbH, 91052 Erlangen**
- **Peter Pflugbeil GmbH, 85604 Zorneding**
- **Sarstedt AG & Co., 51582 Nürnbrecht**

Wednesday, June 6th, 2012
20:15 – 21:00 h

1 Opening lecture:
How many am I – how much is me?
Reflections on the leaky boundaries of individuals
and its consequences in biology and medicine

Prof. Dr. med. Oskar A. Haas
St. Anna Children's Hospital & medgen.at GmbH, Vienna

Thursday, June 7th, 2012
09:15 - 10:45 h

2 Acute leukemias – biology and molecular pathomechanisms I

Chair: Dr. Olaf Heidenreich

2.a Charactarization of oncogenes on chromosome 21 identified by shRNA-based visability screening
Stachorski L, Thangapandi VR, Reinhardt D, Klusmann JH
Hannover

2.b Depletion of RUNX1/ETO in t(8:21)AML cells leads to genomewide changes in transcription factor binding
Martinez N, Plasinska A, Bonifer C, Heidenreich O
Newcastle upon Tyne, Birmingham

2.c The role of PAX5 fusion genes in the pathogenesis of B-cell precursor acute lymphoblastic leukemia (BCP-ALL)
Anderl S, Fortschegger K, Dagmar D, Strehl S,
Vienna

2.d Focal adhesion kinase (FAK) in t(8:21) rearranged acute myeloid Leukaemia (AML)
Prall S, Slack J, Siekmann I, Martinez N, Heidenreich O
Newcastle upon Tyne, Hamburg

2.e Formation of atypical, hyperprofilerating eosinophilic precursors by overexpressing of GATA1s
Maroz A, Henning C, Van Handel B, Mikkola H, Reinhardt D, Klusmann JH
Hannover, Los Angeles

2.f The protein-protein interaction network within human AF4 and AF4-MLL protein complexes
Rössler T, Scholz B, Dingermann T, Marschalek R
Frankfurt

2.a

Characterization of oncogenes on chromosome 21 identified by shRNA-based viability screening

Stachorski L[1], Thangapandi VR[1], Reinhardt D[1], Klusmann JH[1]

[1]Medical School Hannover, Germany

Introduction: Children with trisomy 21 (Down syndrome, DS) are predisposed to develop acute megakaryoblastic leukemia (DS-AMKL). Hypothesizing that the presence of an extra copy of human chromosome 21 (hsa21) is one basal requirement for the onset of DS-AMKL, we previously conducted a shRNA-based viability screening to identify those yet unknown oncogenic candidates.

Methods: We analyzed how candidate oncogenes from the viability screening affect proliferation, self-renewal, apoptosis, colony-forming capacity and differentiation in vitro.

Results: Knockdown of *USP25* selectively mediated proliferation arrest and apoptosis in *GATA1s*-mutated CMK cells, suggesting a synthetic lethal phenotype in conjunction with *GATA1s*. Besides, we identified a gene (*ATP5O*) that blocked megakaryocytic differentiation of K562 and M-07. Knockdown of this gene could overcome this differentiation blockage leading to the acquisition of a megakaryocytic immunophenotype and morphology.

Conclusion: We could delineate the function of multiple putative oncogenes on hsa21 underlining the complex genetic background induced by trisomy 21 during leukemogenesis.

2.b

Depletion of RUNX1/ETO in t(8:21) AML cells leads to genome-wide changes in transcription factor binding

Natalia Martinez1, Anetta Ptasinska2, Constanze Bonifer2and Olaf Heidenreich1.

1 NICR, Newcastle University, UK; 2 Institute of Biomedical Research, Birmingham, UK.

The t(8:21) translocation is present in approximately 15% of all acute myeloid leukemia (AML) cases. This fusion protein is a leukaemia-initiating transcription factor that interferes with RUNX1 function, resulting in a block in differentiation and in the development of AML.

However the molecular details of the mechanism of deregulation are still obscure. It is important to identify RUNX1/ETO target sites at the genome-wide level, to define its individual role in reprogramming gene expression networks. To this end we performed Chip-sequencing and found peaks located in already established RUNX1/ETO target genes as well as in the TERT - CLPTM1L locus.

Since RUNX1 and RUNX1/ETO bind to the same sequence through its RHD domain, it is also important to elucidate how chromatin and RUNX1 occupancy respond to loss of RUNX/ETO binding at its target genes.RUNX1/ETO depletion increased the number of RUNX1 peaks and resulted in an increased number of de novo RUNX1 binding sites. We show that removal of RUNX1/ETO leads to reversal of epigenetic reprogramming and a genome-wide re-distribution of RUNX1 binding.

2.c

The role of PAX5 fusion genes in the pathogenesis of B-cell precursor acute lymphoblastic leukemia (BCP-ALL)

Anderl S, Fortschegger K, Dagmar D, Strehl S

St. Anna Kinderkrebsforschung, CCRI, Children's Cancer Research Institute, Vienna, Austria

PAX5 is an important transcription factor which controls B-lymphocyte development and is a frequent target of somatic mutations in BCP-ALL. In 2-3% of the cases genetic rearrangements lead to the expression of chimeric fusion proteins which are thought to act as aberrant transcription factors that antagonize normal PAX5 function.

The aim of our study is to elucidate potential functional commonalities and differences between the PAX5 fusion proteins PAX5-DACH1, PAX5-DACH2, PAX5-ETV6, PAX5-HIPK1, and PAX5-POM121. The preliminary results of Luciferase assays using PAX5 fusion expression vectors and PAX5 target sequence reporters show that some PAX5 fusion proteins activate PAX5 targets while others do not. These data suggest that PAX5 fusion proteins may have a distinct partner protein-dependent impact on PAX5 target gene expression.

This project is funded by the Austrian Science Fund, FWF P21554-B19.

2.d

Focal adhesion kinase (FAK) in t(8;21) rearranged acute myeloid leukaemia (AML)

Prall S[1], James Slack[1], Ina Siekmann[2], Natalia Martinez[1], Heidenreich O[1]

[1]Northern Institute for Cancer Research, Newcastle upon Tyne, UK; [2]FI Kinderkrebszentrum Hamburg, Germany.

Focal adhesion kinase (FAK) is a non-receptor tyrosine kinase which regulates various cellular processes such as the dynamic of focal adhesion complexes, cell motility, angiogenesis and cell survival. FAK plays a prominent role in various solid tumours, where it is implicated in tumour formation. Increased FAK expression levels generally correlate with their increased invasive potential.Although FAK has been linked to shorter overall survival and enhanced blast migration in AML, the exact role of FAK in haematological diseases remains to be elucidated.

Here we show that siRNA mediated knock-down of AML1/MTG8 leads to a significant reduction of FAK mRNA expression and protein levels. ChIP experiments demonstrated AML1/MTG8 binding to a putative PTK2 (the FAK gene) promoter region 500 bp to 2000 bp upstream of the PTK2 gene. Furthermore application, FAK Inhibitor II, impinges on proliferation of t(8;21)-positive Kasumi-1 and SKNO-1cells.

PTK2 is a direct target gene of AML1/MTG8 which is transcriptionally activated by the latter. Given its important role in solid tumours, FAK might be a relevant target for the treatment of AML.

2.e

Formation of atypical, hyperproliferating eosinophilic precursors by overexpression of GATA1s

Maroz A[1], Hennig C[2], Van Handel B[3], Mikkola H[3], Reinhardt D[1], Klusmann JH[1]

[1]Medical School Hannover, Germany. [2]University of California Los Angeles, USA

Transcription factor GATA1 is essential for the development of megakaryocytes, erythrocytes and eosinophils. GATA1-mutations (GATA1s) are associated with Down syndrome (DS) transient leukemia (TL).

Here, we report a series of DS-TL patients with eosinophilia that carry the same GATA1s-mutation in the sorted eosinophils as in the leukemic blasts. To explore the effect of GATA1s on myeloid differentiation in the human context, we established a lentiviral vector system for simultaneous downregulation of endogenous GATA1 and ectopic expression of GATA1s/GATA1 in hematopoietic stem and progenitor cells (HSPCs) from human fetal livers. Both, GATA1 or GATA1s fully blocked monocytic differentiation and impaired neutrophilic differentiation *in vitro*. We observed accumulation of atypical eosinophilic precursors with basophilic characteristics and formation of atypical myeloid colonies. Only GATA1s lead to the hyperproliferation of these cells.

Thus, our observations explain the frequently observed hypereosinophilia in DS-TL patients as a part of the leukemic clone and the role of mutated GATA1s in this myeloid phenotype.

2.f

The protein-protein interaction network within human AF4 and AF4-MLL protein complexes

Rössler T, Scholz B, Dingermann T, Marschalek R

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We have recently established the composition and function of the human AF4 and AF4-MLL fusion protein complex (Benedikt et al., 2011). The AF4 complex is responsible for transcriptional elongation, while the AF4-MLL complex is causing ectopic transcription, ectopic histone signatures and was shown to induce proB ALL in a mouse model. The composition of both complexes has been eludidated by using affinity-purification from human cells, nano LC-MS/MS analyses, Western blot and co-IP experiments. Functional analysis was performed by using different in vitro and in vivo assays. Here, we will present our preliminary data on the dissection of the protein-protein interaction network of complex components. This work has been done by subcloning about 35 protein-coding fragments in bait and prey vectors, and to use these vectors in combination with the yeast-2-hybrid system to unravel the AF4-N protein interactome.

This work is supported by grant Ma 1876/10-1 from the Deutsche Forschungsgemeinschaft.

Thursday, June 7th, 2012
11:15 – 12: 15 h

3 Stem cell research, leukemogenesis and transplantation

Chair: Dr. Lüder Hinrich Meyer

3.a Lentiviralmarking as a tool to investigate the clonal complexity and evolution of ALL
Elder AK, Heidenreich O, Vormoor HJ
Newcatle upon Tyne,

3.b Haematopoietic stem cell survival and transplantation efficacy is limited by the BH3-only proteins Bim and Bmf
Bertele D, Labi V, Woess C, Grothe G, Schwemmers S, Pahl HL, Geley S, Kunze M, Niemeyer CM, Villunger A, Erlacher M
Freiburg, Innsbruck

3.c Timely controlled T cell receptor expression against a leukemia-associated antigen for the co-transplantation of MHC-mismatched T-cell precursors into hematopoietic stem cell (HCT) recipients
Hoseini S, Hapke M, Herbst J, Heinz N, Schiedlmeyer B, Krüger A, Sauer M
Hannover

3.d AML1/ETO Confers a Mutator Phenotype in AML
Forster V, Beyerle A, Heidenreich O, Allan J
Newcastle upon Tyne

3.a

Lentiviralmarking as a tool to investigate the clonal complexity and evolution of ALL

Elder AK, Heidenreich O, Vormoor HJ

Northern Institute for Cancer Research, Newcastle University, UK

Introduction: There remains significant controversy over the applicability of cancer stem cell models to acute lymphoblastic leukaemia. Recent data suggest that the propagating compartment undergoes a clonal evolution process, leading to the co-existence of several different subclones.

Methods: We aim to provide a comprehensive picture of the clonal complexity and evolution of ALL by lentivirally marking individual leukaemia cells with heritable genetic markers. This will be achieved using a cellular barcoding strategy and analysis of lentiviral insertion sites. This will allow us to address numerous questions, including assessing the number of clones that contribute to leukaemia following grafting into NSG mice and the Darwinian fitness of matched presentation and relapse samples.

Results: The use of ligation mediated PCR successfully identifies integration sites in lentivirally transduced leukaemia cells. This also facilitates investigations into whether lentiviral marking leads to the development of dominant clones through insertional mutagenesis.

Conclusions: Lentiviral marking of leukaemic stem cells is a powerful tool to investigate the in vivo development of leukaemia.

3.b

Haematopoietic Stem Cell Survival and Transplantation Efficacy is Limited by the BH3-only Proteins Bim and Bmf

Daniela Bertele[1], Verena Labi[2], Claudia Woess[2], Gesina Grothe[1], Sven Schwemmers[3], Heike L. Pahl[3], Stephan Geley[4], Mirjam Kunze[5], Charlotte M. Niemeyer[1], Andreas Villunger[2] and Miriam Erlacher[1].

[1]Department of Pediatrics and Adolescent Medicine, Division of PediatricHematology and Oncology, University Hospital of Freiburg; [2]Division of Developmental Immunology, Biocenter, Innsbruck Medical University; [3]Section of Experimental Anesthesiology, Center for Clinical Research, University Hospital Freiburg; [4]Division of Molecular Pathophysiology, Biocenter, Innsbruck Medical University; [5]Department of Obstetrics and Gynecology, University Hospital Freiburg, Germany.

Cell death occurring in haematopoietic stem cells (HSC) is critically involved in limiting successful stem cell transfer. It is known that HSC homeostasis is regulated by anti-apoptotic Bcl-2 family members, but little is known about the role of their antagonists, the “BH3-only” proteins. We determined the consequences of BH3-only protein depletion on HSC survival *in vitro* and *in vivo*. Thereby, we identified two BH3-only proteins, Bim and Bmf, as regulators of HSC survival. *Bim*$^{-/-}$ or *bmf*$^{-/-}$ HSC performed better during early engraftment and long term reconstitution of the haematopoietic system. In competitive transplantation experiments, wild type HSC were readily displaced by *bim*$^{-/-}$ or *bmf*$^{-/-}$ HSC as early as 10 days after transplantation. Moreover, in the absence of Bim, lower numbers of HSC were required for successful host reconstitution.

Finally, we provide evidence that Bim and BMF have conserved functions between mice and men. Downregulation of either protein in cord blood derived huCD34^{+} cells lead to a superior reconstitution of *rag2*$^{-/-}$*γc*$^{-/-}$mice. We therefore believe, that Bim and Bmf may be attractive therapeutic targets to increase HSC survival and transplantation efficacy.

3.c

Timely controlled T cell receptor expression against a leukemia-associated antigen for the co-transplantation of MHC-mismatched T-cell precursors into hematopoietic stem cell (HCT) recipients

S. Hoseini[1], M. Hapke[1], J. Herbst[1], N. Heinz[2], B. Schiedlmeyer[2], A. Krüger[3], and M. Sauer[1]

Departments of (1) Pediatric Hematoloogy/Onkology, (2) Experimental Hematology, and (3) Experimental Immunology. Medizinische Hochschule Hannover, Germany.

Adoptive transfer (AT) of TCR-gene modified T cells has been associated with a couple of draw-backs. 1. Rigorous *in vitro* stimulation required for TCR-gene transduction can profoundly impact in vivo function after AT, 2. TCR-chain mispairing can cause potentially life threatening autoreactivity, and 3. HLA-matched T cell donors are required. Here we present a system designed to transduce stem cells with an anti-leukemic TCR-gen whose expression is regulated by a tetracycline-response-element (TRE). The new vector was primarily evaluated on murine T 58 cells and human Jurkat cells. The addition of doxycycline resulted in prompt expression of the TCR and its respective reporter in vitro. Importantly, removal of the agent resulted in complete downregulation within two days. These switch on/off cycles could be repeated multiple times over several weeks showing the stability of the system. Consecutively we generated T-lineage committed lymphoid precursor cells (predominantly with a phenotype comparable to double CD4 and CD8 negative (DN)2 and DN3 thymocytes) using the bone-marrow-derived OP9 cells that express the Notch ligand DL1. T cell precursors were cultured from Lin^-, Sca 1^+, c-kit^{hi} stem cells using Flt3-ligand and IL-7. After transduction with our new plasmid T-cell precursors carrying the gene of interest were generated. This is relevant since it will allow co-transfer of third party precursors avoiding thymic negative selection of leukemia TCR-expressing immature precursors.

3.d

AML1/ETO Confers a Mutator Phenotype in AML

Forster V, Beyerle A, Heidenreich O, Allan J

Northern Institute for Cancer Research, Newcastle University, UK

The translocation t(8;21), resulting in the AML1/ETO (AE) fusion gene is the most common cytogenetic abnormality in acute myeloid leukaemia(AML). In animal models, AE expression is insufficient for leukaemogenesis with further mutations required for leukaemic transformation.

Ectopic expression of AE in TK6 and HL60 cell lines results in ~2-fold down-regulation of the gene OGG1, a DNA glycosylase which removes damaged guanine bases from DNA. Knock-down of AE in Kasumi-1 increased OGG1 substantially, providing strong evidence that AE directly regulates OGG1.

TK6 AE were cloned to give varying AE expression and analysed for mutation of TK or PIGA. AE conferred a 2-fold higher background TK mutation frequency (MF) compared to controls and a >2 fold higher PIGA MF, suggesting that AE confers a moderate mutator phenotype even without exposure to genotoxic agents. When treated with doxorubicin or ionising radiation to induce mutations, AE TK6 clones had ~4 fold higher MF in comparison to controls ($p<0.001$).

We show that AE confers a mutator phenotype, associated with downregulation of OGG1. We also suggest a mechanism by which AE pre-leukaemic haematopoetic cells acquire additional mutations, leading to leukaemic transformation.

Thursday, June 7th, 2012
14:00 – 15:00 h

4 Biology of neuroblastoma

Chair: Prof. Roland Kappler

4.a The role of TrkA expression in checkpoint activation and DNA double strand break repair in neuroblastoma cells
Rudolf I, Lindner S, Schramm A, Eggert A, Kuhfittig-Kulle S
Essen

4.b LIN28B drives neuroblastoma oncogenesis through let7-MYCN signaling
Lindner S, Molenaar J, Thor T, Sprüssel A, Caron HN, Versteeg R, Schramm A, Eggert A, Schulte JH
Essen, Amsterdam

4.c The JARID1C histone demethylase is upregulated in aggressive Neuroblastomas independent of MYCN amplification
Fielitz K, Schowe B, Schulte JH, Vandesompele J, Mestdagh P, Eggert A, Morik K, Schramm A
Essen, Dortmund, Ghent

4.d Investigation of Schwann cells secreted factors inhibiting aggressive neuroblastoma growth
Weiss T, Taschner-Mandl S, Brunner C, Ambros IM, Ambros PF
Vienna

4.a

The role of TrkA expression in checkpoint activation and DNA double strand break repair in neuroblastoma cells

I Rudolf, S Lindner, A Schramm, A Eggert, S Kuhfittig-Kulle

University Children`s Hospital Essen, Germany

Background: High expression of the receptor tyrosine kinase TrkA is associated with favorable outcome in neuroblastoma. TrkA has been shown to enhance the capacity of DSB repair in SY5Y cells and upregulation of a major factor in NHEJ, XRCC4, has been demonstrated in SY5Y cells stably expressing TrkA.

Methods: Stably transfected SY5Y cells carrying an inducible vector for TrkA were used to analyze its effect on cell viability, DNA-damage-induced cell death and the DNA repair capacity in response to ionizing radiation (IR) *in vitro*.

Results: After moderate doses of IR, proliferation of TrkA-positive SY5Y cells was comparable to proliferation of non-irradiated cells, while proliferation of TrkA-negative cells was greatly diminished. Significant changes in cell cycle distribution were shown in the TrkA-expressing cells compared to non-transfected cells. PARP expression, but not PARP cleavage was increased in TrkA-expressing cells.

Conclusion: The upregulation of PARP in TrkA-expressing cells suggests TrkA involvement in the regulation of this protein. Proliferation of TrkA-positive cells after IR point to a protective effect of TrkA against IR-induced cell death, but underlying mechanisms need to be studied in greater detail.

4.b

LIN28B drives neuroblastoma oncogenesis through let7-MYCN signaling

Lindner S[1], Molenaar J[2], Thor T[1], Sprüssel A[1], Caron HN[2], Versteeg R[2], Schramm A[1], Eggert A[1], Schulte JH[1]

[1]Department of Paediatric Oncology and Hematology, University Children's Hospital Essen, Germany; [2]Dept. of Oncogenomics, Academic Medical Center, Amsterdam, The Netherlands.

LIN28b is overexpressed in many malignancies, including neuroblastoma (NB). LIN28b represses let-7 miRNAs which target MYC, RAS and MYCN. We here describe for the first time that LIN28b has the potential to initiate NBs.

We established a genetically engineered mouse model overexpressing murine Lin28b in the neural crest and its derivates. The spontaneously arising tumors were analysed using IHC, western blot and RT-qPCR.

Tumors originated from locations also common for human NB (e.g. adrenal). All tumors consisted of small round blue cells and expressed TH, DBH and Phox2b, confirming them to be NBs. While MYCN was upregulated in these tumors, let-7 miRNAs were downregulated.

We here show that Lin28b is a bona fide oncogene in NB that regulates MYCN via let-7 miRNAs. Inhibition of Lin28b or re-expression of let-7 miRNAs may be useful therapeutic approaches in LIN28b driven NBs.

4.c

The JARID1C histone demethylase is upregulated in aggressive neuroblastomas independent of *MYCN* amplification

Fielitz K[1], Schowe B[2], Schulte JH[1], Vandesompele J[3], Mestdagh P[3], Eggert A[1], Morik K[2], Schramm A[1]

[1]University Childrens Hospital Essen; [2]TU Dortmund; [3]CMGG, Ghent, Belgium.

Background: Defining reliable risk factors for progression and outcome in advanced stage neuroblastoma (NB) patients not harboring a *MYCN* amplification is still challenging.

Methods: Expression profiles were generated from 113 primary NBs on Affymetrix microarrays. Genes associated with patient outcome but independent of known risk factors were identified using correlation analysis.

Results: Expression of JARID1C was significantly elevated in primary NBs from patients who later suffered relapse, independent of *MYCN* amplification status. The JARID1C protein was detectable in all NB cell lines analyzed. Cell viability and cycle were analyzed after JARID1C knockdown using siRNA in SHEP and IMR 5. Analyses of H_3K_4 methylation status following siRNA-mediated JARID1C knock-down confirmed inhibition of JARID1C function. JARID1C knockdown significantly decreased cell viability, induced morphological changes, and increased the fraction of apoptotic cells.

Conclusion: We identified JARID1C as a novel and independent marker of aggressive NB that may be linked to NB tumor maintenance.

4.d

Investigation of Schwann cells secreted factors inhibiting aggressive neuroblastoma growth

Weiss T1, Taschner-Mandl S1, Brunner C1, Ambros IM1, Ambros PF1

1Children´s Cancer Research Institute, St. Anna Kinderkrebsforschung, 1090 Vienna, Austria

Introduction: Neuroblastoma (NB) is the most common solid neoplastic disease during infancy. In contrast to aggressive NBs, maturing/mature NBs are associated with a favourable prognosis and harbour a special characteristic, a non-neoplastic Schwann cell (SC) stroma.

Previous Results: Published data suggest that reciprocal signalling between SCs and NB cells impair NB growth by differentiation, apoptosis and proliferation-stop of NB cells accompanied by decreased vascularity. In vivo, only genetically favourable NBs undergo spontaneous maturation, however, previous results showed that aggressive NB cells with unfavourable genetics are responsive to SCs when 'artificial' contact is enabled by co-cultivation or SC conditioned media causing differentiation, apoptosis and reduced proliferation of NB cells. Hence, SC secreted factors promoting these effects are from obvious interest for NB treatment but are largely unexplored in these tumours.

Aim: We want to identify the SC secreted factors able to induce growth inhibition in aggressive NB cells and propose to analyse the secretory protein profile of SCs upon co-cultivation with aggressive NB cell lines to reveal candidate factors.

Thursday, June 7th, 2012
15:30 – 16:45 h

5 Acute leukemias, treatment response and outcome

Chair: Prof. Reinhard Schneppenheim

5.a Diverse receptor tyrosine kinase dependent signaling networks determine profileration and survival in childhood acute lymphoblastic leukemia
Dierck K, Prall S, Siekmann I, Behlich AS, Beck F, Trochimiuk M, Strauss J, Gottschling K, Kosh M, Müller J, Harder L, Kenkhar M, Sternsdorf T, Jeremias I, Sickmann A, Nollau P, Horstmann MA
Hamburg, Dortmund, Munich

5.b A mathematical approach to data evaluation with focus on prediction of minimal residual disease in pediatric ALL
Torge A, Zimmermann M, Möricke A, Köhler R, Schrauder A, Bartram CR, Schrappe M, Stanulla M
Kiel, Hannover, Heidelberg

5.c Genetic abberations involved in glucocorticoid-mediated response of *ETV6/RUNX1*-positive leukemia
Bastelberger S, Grausenburger R, Eckert C, Stanulla M, Panzer-Grümayer R
Vienna, Berlin, Kiel

5.d MRD-quantification during treatment of *ETV6-RUNX1* positive ALL relapses using the genomic breakpoint of the fusion gene
Hoffmann J, Metzler M, von Stackelberg A, Eckert C,
Berlin, Erlangen

5.e Application of genomic breakpoints as molecular markers in pediatric leukemia and sarcoma
Krumbholz M, von Goessel H, Karl M, Berger M, Metzler M,
Erlangen

5.a

Diverse receptor tyrosine kinase dependent signaling networks determine proliferation and survival in ALL

Dierck K[1], Prall S[1,], Siekmann I[1], Behlich AS[1], Beck F[3], Trochimiuk M[1], Strauss J[1], Gottschling K[1], Kosh M[1], Müller J[1], Harder L[1], Kenkhar M[4], Sternsdorf T[1], Jeremias I[5], Sickmann A[3], Nollau P[4], Horstmann MA[1,2]

[1]Research Institute Children's Cancer Center Hamburg, Hamburg; [2]Clinic of Pediatric Hematology and Oncology, University Medical Center Hamburg-Eppendorf, Hamburg; [3]Department of Bioanalytics, ISAS, Dortmund; [4]Institute of Clinical Chemistry, University Medical Center Hamburg-Eppendorf; Hamburg [5]Helmholtz Center, Munich

The balanced regulation of complex signaling networks plays an important role in many cellular processes such as proliferation and apoptosis whereas aberrant signaling is a hallmark of many diseases. Receptor tyrosine kinases (RTK) initiate phosphotyrosine dependent signaling pathways which are often deregulated in various malignancies. We assessed the functional importance of RTK dependent signal transduction in acute lymphoblastic leukemia (ALL). Phosphoproteomic studies applying SH2 domain profiling showed a heterogeneous state of tyrosine phosphorylation in primary ALL. The extensive analysis of RTK protein expression and activation state in primary ALL cells, ALL cell lines, and normal hematopoietic cells revealed distinct and aberrant RTK signatures. Receptor activation specifically modulated downstream signaling and contributed to proliferation of ALL cells while RTK knockdown substantially impaired proliferation and survival of leukemic blasts *in vitro* and *in vivo* in a mouse xenotransplant model. The identification of specifically regulated phosphoproteins by mass spectrometry led to the identification of highly diverse RTK dependent signaling networks.

5.b

A mathematical approach to data evaluation with focus on prediction of minimal residual disease in pediatric ALL

Torge A[1], Zimmermann M[2], Möricke A[1], Köhler R[3], Schrauder A[1], Bartram CR[3], Schrappe M[1], Stanulla M[1]

[1]Department of Pediatrics, University Hospital Schleswig-Holstein, Kiel Germany; [2]Pediatric Hematology and Oncology, Hannover Medical School, Hannover, Germany; [3]Department of Human Genetics, University of Heidelberg, Heidelberg, Germany

In pediatric acute lymphoblastic leukemia (ALL), the most important prognostic factor for tailoring the intensity of treatment according to the probability of failure is the evaluation of minimal residual disease (MRD) at defined timepoints.

One drawback of MRD analyses is that results are not available at diagnosis to guide selection of the optimal treatment intensity already early on. In trial ALL BFM 2000, multi-level patient data were collected and are now used to develop a mathematical approach to predict MRD. For this task, mathematical methods like neuronal networks or Fuzzy logic are applied and rigorously tested regarding their capability to accurately predict MRD.

Modeling approaches and first results will be presented at the meeting.

5.c

Genetic aberrations involved in glucocorticoid-mediated response of ETV6/RUNX1-positive leukemia

Bastelberger Stephan[1], Grausenburger R[1], Eckert C[2], Stanulla M[3], Panzer-Grümayer R[1]

[1]St. Anna Kinderkrebsforschung, Children's Cancer Research Institute, Vienna, Austria; [2]Department of Pediatric Oncology/Hematology, Charité Universitätsmedizin Berlin, Berlin, Germany; [3]Department of Pediatrics, University Medical Centre Schleswig-Holstein, Kiel, Germany

The t(12;21) is the most frequent chromosomal translocation in childhood acute lymphoblastic leukemia (ALL), generating the *ETV6/RUNX1* (*E/R*) fusion gene, and generally associated with excellent initial treatment response. Relapses, however, occurring in up to 20% of cases, are often associated with drug resistance and dismal outcome. We recently reported on the particularly high frequency of genomic aberrations in genes involved in glucocorticoid (GC)-mediated apoptosis in *E/R*-positive relapsed leukemias (Kuster et al., Blood 2011). Here, we selected *NR3C1*, encoding the GC receptor (GR), for further validation and functional analysis using two approaches. i) Functional assessment of *NR3C1* regulation by restoring the GR expression in *NR3C1*-deleted and GC-resistant E/R-positive leukemia cell lines and repression of GR signalling by a specific antagonist in wt *NR3C1* and GC-sensitive cell lines. Results obtained so far support our assumption that GR levels are critical for GC sensitivity in E/R-positive ALL. ii) Genome-wide copy number analysis and mutation screening of *NR3C1* of primary ALL samples including PPR and relapse cases. Upon completion of these analyses, deletion and mutation status will be related to clinical outcome. The study is expected to shed light on potential relapse and resistance mechanisms of this leukemia subgroup, ultimately leading to new treatment strategies.

5.d

MRD-quantification during treatment of *ETV6-RUNX1* positive ALL relapses using the genomic breakpoint of the fusion gene

Hoffmann J[1], Metzler M[2], von Stackelberg A[1], Eckert C[1]

[1] Charité Universitätsmedizin Berlin; [2] Universität Erlangen, Germany.

T-cell receptor (TCR)/Immunoglobulin(IG)-gene rearrangements used as minimal residual disease (MRD) markers in acute lymphoblastic leukaemia (ALL) can describe different subclones at initial/relapse diagnosis because leukaemic clones are exposed to influences of clonal evolution and selection during/after treatment.

We aimed at investigating whether the genomic breakpoint of the fusion gene *ETV6-RUNX1* (E/R) is a suitable MRD-marker.

Patients with an E/R positive first ALL relapse were included in the study (n=76). Identification and sequencing of the breakpoint was performed using nested multiplex long-range PCR. Clone specific primer/probe sets were designed, tested, and used for MRD-quantification (n=279 samples).

In 91% of patients (43/47) a sensitivity of 1E-04/-05 was reached. The quantitative data correlated well with the TCR/IG-results (>1/2 log-step difference in 34/279). The genomic breakpoint sequence was identical between different disease stages.

The genomic breakpoint of the fusion gene reveals as a very sensitive, stable MRD-marker and is therefore excellently suitable for MRD-quantification in E/R positive ALL.

5.e

Application of genomic breakpoints as molecular markers in pediatric leukemia and sarcoma

Krumbholz M, von Goessel H, Karl M, Berger M, Metzler M

Department of Pediatrics, University Hospital Erlangen, Germany.

Chromosomal translocations and the resultant fusion genes are recurrent tumor-specific markers of a large spectrum of pediatric tumors, particularly leukemia and sarcoma. The use of DNA fusion sequences as genomic minimal residual disease markers is often hampered by the extended size of breakpoint cluster regions, difficult to cover by conventional PCR applications. We developed and optimized nested multiplex long-range PCR assays for the identification of frequent fusion genes involved in ALL (*TEL-AML1, E2A-PBX1, MLL, BCR-ABL1*), AML (*AML1-ETO, MLL*), CML (*BCR-ABL1*), ALCL (*NPM-ALK*), Ewing's sarcoma (*EWS-FLI1, EWS-ERG*) and rhabdomyosarcoma (*PAX3-FKHR, PAX7-FKHR*). The patient-specific fusion sites represent high sensitivity DNA markers for absolute quantification of tumor cells independent of gene expression and resistant to marker-loss by clonal evolution. In addition, a detailed characterization of genomic breakpoints allows insights in breakpoint initiation mechanisms.

Thursday, June 7th, 2012
17:00 – 17:45 h

6 Brain tumors I – genetics and biology

Chair: Prof. Ivo Leuschner

6.a Nuclear exclusion of TET 1 is associated with loss of 5-hydroxmethylcytosine in *IDH1* wildtype gliomas
Müller T, Gessi M, Waha A, Isselstein LJ, Luxen D, Freihoff D, Freihoff J, Becker A, Simon M, Pietsch T, Waha A
Bonn

6.b Molecular anaylsis of Gremlin in glimoas
Isselstein LJ, Gessi M, Müller T, Luxen D, Freihoff D, Freihoff J, Becker A, Simon M, Pietsch T, Waha A, Waha A
Bonn

6.c Molecular analysis of MTSS1 in gliomas
Luxen D, Gessi M, Isselstein LJ, Müller T, Freihoff D, Freihoff J, Becker A, Simon M, Pietsch T, Waha A, Waha A
Bonn

6.a

Nuclear exclusion of TET1 is associated with loss of 5-hydroxymethylcytosine in *IDH1* wildtype gliomas

Müller T[1], Gessi M[1], Waha A[1],Isselstein LJ[1], Luxen D[1], Freihoff D[1], Freihoff J[1], Becker A[1], Simon M[2], Pietsch T[1] and Waha A[1]

[1]Department of Neuropathology, [2]Department of Neurosurgery, University of Bonn, Germany.

IDH1 mutations are associated with a CpG island methylator phenotype in glioblastomas. Cells harboring *IDH1* mutations produce 2-hydroxyglutarate inhibiting TET enzymes involved in the oxidation of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC).

Glioma tissues and cell lines were investigated by RT-PCR, immunohistochemistry and pyrosequencing for the expression of TET enzymes, 5hmC content and *IDH1* status respectively.

61% of gliomas revealed no immunoreactivity for 5-hmC and no correlation was observed between *IDH1* mutations and loss of 5hmC. The subcellular localization of TET1 showed remarkable differences. *IDH1* mutations were significantly correlated with nuclear accumulation of *TET1*. 70% of 5-hmC negative gliomas showed either exclusive or dominant cytoplasmic expression of TET1 suggesting that its nuclear exclusion may influence the 5hmC content of cells.

6.b

Molecular analysis of Gremlin in gliomas

Isselstein LJ[1], Gessi M[1], Müller T[1], Luxen D[1], Freihoff D[1], Freihoff J[1], Becker A[1], Simon M[2], Pietsch T[1] Waha A[1] and Waha A[1]

[1]Department of Neuropathology; [2]Department of Neurosurgery, University of Bonn, Germany.

Gremlin was identified as a regulator of growth and development in Xenopus laevis and a transcript downregulated in mos transformed rat cells.

Hypermethylation and expression of Gremlin was studied in gliomas by pyrosequencing and realtime RT-PCR in 63 gliomas, 6 GBM cell lines and 7 stem cell enriched primary GBM cell cultures. Structural alterations were investigated by SNP analysis and high resolution melting studies. The capacity of focus formation was studied on transiently transfected GBM cells.

Gliomas showed remarkably reduced mRNA-levels of Gremlin and excessive hyper-methylation compared to normal brain tissues. 5 GBM samples revealed LOH at the investigated SNPs. In addition we observed missense mutations of codon 35 (P35A, P35L). Focus formation was significantly reduced in transfected glioblastoma cell lines.

These findings argue for a contribution of epigenetic silencing of Gremlin to the molecular pathology of gliomas.

6.c

Molecular analysis of MTSS1 in gliomas

Luxen D[1], Gessi M[1], Isselstein LJ[1], Müller T[1], Freihoff D[1], Freihoff J[1], Becker A[1], Simon M[2], Pietsch T[1] Waha A[1] and Waha A[1]

[1]Department of Neuropathology; [2]Department of Neurosurgery, University of Bonn, Germany.

In a genome-wide methylation analysis, the metastasis suppressor-1 (*MTSS1*) gene was identified as a novel gene hypermethylated in gliomas. MTSS1 interacts with actin filaments and promotes Gli dependent transcriptional regulation.

Here we investigate *MTSS1* methylation, allelic loss and expression in 63 gliomas and 6 glioma cell lines by pyrosequencing, realtime RT-PCR and IHC. MTSS1 was overexpressed in glioma cell lines to study localization and biological function of the protein.

MTSS1 hypermethylation was frequently detected in anaplastic astrocytomas and secondary GBM where it correlated with *IDH1* mutational status. Only few informative gliomas showed allelic loss of one *MTSS1* allele. Epigenetic silencing of *MTSS1* was confirmed by treatment of glioma cell lines with 5-aza2'deoxycytidine. Transfected cells showed staining of focal contact structures suggesting a role of MTSS1 in adhesion to the extracellular matrix.

Thursday, June 7th, 2012
17:45 – 19:00 h

7 **Special anniversary lectures** (in German language):
Pädiatrische Onkopathologie – Erinnerungen und Erfahrungen
Prof. Dr. med. Dieter Harms
University Hospital Schleswig-Holstein, Campus Kiel

40 Jahre Wilsede Meetings – Weiter mit Science Channel Wilsede
Prof. Dr. med. Rolf Neth
University Hospital Hamburg-Eppendorf

Friday, June 8th, 2012
09:15 – 10:45 h

8 Acute leukemias – biology and molecular pathomechanisms II

Chair: Dr. Jan-Henning Klusmann

8.a Using the sleeping beauty system to investigate different MLL fusion genes
Karl K, Kowarz E, Dingermann T, Marschalek R
Frankfurt

8.b Molecular characterization of lincRNAs in Down syndrome associated leukemia
Streltsov A, Emmrich S, Engeland F, Klusmann JH
Hannover

8.c Mechanisms of ETV6/RUNX1-mediated gene regulation in leukemia
Nassimbeni C, Fuka G, Morak M, Grausenburger R, Panzer-Grümayer R
Vienna

8.d Structural and functional analysis of Taspase1
Engelbrecht C, Sabiani S, Dingermann T, Marschalek R
Frankfurt

8.e Identification of dysregulated signal transduction pathways – Case study of biphenotypic acute leukemia (BAL)
Selle L, Heiden T, Bommer C, Klopocki E, Gerling A, Klehm U, Robinson N, Teubner B, Trotier F, Trimborn M, Türkmen S, Seeger K
Berlin

8.f Investigating the role miR-139 and miR-582 in AML1-ETO associated leukemia
Engeland F, Emmrich S, Kuipers J, van den Heuvel-Eibrink MM, Reinhardt D, Klusmann JH
Hannover, Rotterdam

8.a

Using the sleeping beauty system to investigate different MLL fusion genes

Karl K, Kowarz E, Dingermann T, Marschalek R

Inst of Pharm Biology, Goethe-University, Max-von-Laue-Str. 9, 60438 Frankfurt/Main, Germany.

The MLL recombinome has expanded to 73 fusion partners over the last years (Meyer et al., 2009). Most of these MLL fusions have never been functionally tested. In addition, about 20% of patients display complex MLL rearrangements involving 3 or 4 recombination partner genes. This led to a collection of nearly 100 reciprocal MLL fusions that have been identified at our diagnostic center. Here we present our preliminary data on our systematic approach to functionally characterize yet unknown direct and reciprocal MLL fusion genes. For the purpose of our studies we have developed an universal vector system for direct and reciprocal MLL fusions by using retroviral, lentiviral or sleeping beauty based vector systems. Here, we will focus on our experiences using the sleeping beauty technology for the creation of stable cell lines and will present our first results obtained with the yet tested MLL fusion genes.

This work is supported by grant DKS 2011.09 from the Deutsche Kinderkrebsstiftung e.V.

8.b

Molecular characterization of lincRNAs in Down syndrome associated leukemia

Streltsov A[1], Emmrich S[1], Engeland F[1], Klusmann JH[1]

[1]Medical School Hannover, Germany

We analyzed the long intervening non-coding RNA (lincRNA) host genes (MIR100HG, LINC00478 and LINC00085) of the mir-99~125 clusters. Those miRNA clusters reflect the phylogenetically most ancient miRNA clusters with important roles in hematopoietic stem and progenitor cell (HSPC) homeostasis and the development of acute megakaryoblastic leukemia (AMKL).

By qRT-PCR-profiling, we could show that MIR100HG, LINC00478 and LINC00085 are overexpressed in AMKL cell lines (Meg-01, CMK, CMY) in contrast to other leukemic cell lines (K562, THP-1, Kasumi). For gain and loss of function studies of lincRNAs, we designed and evaluated two lentiviral vector systems. Upon shRNA-mediated knockdown of MIR100HG and LINC00478 in AMKL cell lines we could show a growth disadvantage (competition assay), proliferation arrest (BrdU incorporation), decreased self-renewal (methocult), and altered differentiation (immunophenotyping).

In conclusion, our data indicate that the lincRNAs MIR100HG and LINC00478 exert oncogenic effects in AMKL. Future murine transplantation experiments and molecular biological assays will provide deeper insights into the function of those lincRNAs.

8.c

Mechanisms of ETV6/RUNX1-mediated gene regulation in leukemia

Nassimbeni C[1], Fuka G[1], Morak M[1], Grausenburger R[1], Panzer-Grümayer ER[1]

[1]Children´s Cancer Research Institute, St. Anna Kinderkrebsforschung, 1090 Vienna, Austria

The t(12;21)(p13;q22) chromosomal translocation, which generates the ETV6/RUNX1 (E/R) fusion gene, is present in about 20% of childhood acute lymphoblastic leukemia (ALL). It leads to a chimeric transcription factor consisting of the N-terminal region of ETV6 and almost the entire RUNX1 protein. E/R is under the transcriptional control of the *ETV6* promoter and regulates transcription via the DNA-binding RUNT homology domain of RUNX1. So far, experimental evidence suggested that E/R acts as a constitutive repressor of RUNX1 target genes by aberrantly recruiting transcriptional repressors. This notion, however, has been challenged by recent publications, which include findings of our group (Fuka et al., PlosOne 2011), showing that E/R also up-regulates RUNX1 target genes. These findings suggest a more complex orchestration of E/R target gene regulation than previously assumed.

To shed light on the various mechanisms of genome-wide E/R target gene regulation in leukemia, we are currently establishing a conditional E/R-expression system in a leukemic model cell line that will be used for comprehensive and integrative analyses of DNA binding and gene regulation, the identification of affected pathways and also of transcription factors that co-operate with E/R on target gene promoters. In this talk the outline and current state of the project will be presented.

8.d

Structural and functional analysis of Taspase1

Engelbrecht C, Sabiani S, Dingermann T, Marschalek R

Inst of Pharm Biology, Goethe-University, Max-von-Laue-Str. 9, 60438 Frankfurt/Main, Germany.

Taspase1 is an endoprotease that hydrolyzes the human MLL and the oncofusion protein AF4-MLL at distinct cleavage sites (CS1 and CS2). It has been demonstrated that both protein complexes require Taspase1 for protein stability and proper function. In terms of t(4;11) leukemia, Taspase1 represents a conditional oncoprotein because Taspase1 cleaves AF4-MLL to form a high molecular weight complex that causes proB ALL. Therefore, we are aiming to identify potential inhibitors against Taspase1. Unfortunately, any efforts to target the enyzmatic center of Taspase1 have failed so far. For the purpose of our studies, we have performed site-directed mutagenesis to dissect all the molecular steps which are necessary to activate Taspase1. Besides dimerization, autoproteolytic cleavage is a critical step that makes Taspase1 active. Our work has led to a working model on how Taspase1 can be allosterically inhibited. We will present our preliminary data and discuss future directions.

This work is supported by grant 107819 from the Deutsche Krebshilfe.

8.e

Identification of dysregulated signal transduction pathways – Case study of biphenotypic acute leukemia (BAL)

Selle L[1], Heiden T[1], Bommer C[2], Klopocki E[2], Gerling A[2], Klehm U[2], Robinson N[2], Teubner B[2], Trotier F[2], Trimborn M[2], Türkmen S[2], Seeger K[1]

[1]Klinik für Pädiatrie m.S. Onkologie/Hämatologie/KMT, Charité - Universitätsmedizin Berlin CVK

[2]Institut für Medizinische Genetik, Charité - Universitätsmedizin Berlin CVK

Introduction: Improved detection of molecular mechanisms in leukemia with novel diagnostic techniques enables new opportunities for targeted therapy. BALs show in their majority complex chromosomal karyotype, as found in a BAL obtained from a 6 year old child. Analyses defining chromosomal rearrangements, genomic imbalances and gene expression changes identifying dysregulated signal transduction pathways.

Material and Methods: In addition to routine diagnostics, molecular (cyto)genetic techniques such as FISH, aCGH, gene sequencing, qPCR and genome-wide oligonucleotide microarray analysis were used to characterize blasts from initial disease and relapse.

Results and Discussion: Pronounced clonal diversity and genetic instability were confirmed, common trait appears to be *K-RAS* amplification and a novel *TCF3-ZNF384* fusion. Data analyses revealed different candidate cancer drivers and suggested a second-line-therapy with the multikinase inhibitor sorafenib.

8.f

Investigating the role of miR-139 and miR-582 in AML1-ETO associated leukemia

Engeland F[1], Emmrich S[1], Kuipers J[2], van den Heuvel-Eibrink MM[2], Reinhardt D[1], Klusmann JH[1]

[1]Medical School Hannover, Germany; [2]Erasmus MC, Rotterdam, The Netherlands

Introduction: The translocation t(8;21) (fusion protein AML1-ETO) can be found in 5-12% of all cases with AML. Here, we investigated the role of miRNA during the development of AML1-ETO associated leukemogenesis.

Results: miR-139 und miR-582 are downregulated in patients with t(8;21). In gain of function studies, we observed a significant reduction of cell proliferation and colony-forming capacity in Kasumi-1 and SKNO-1 cells. MiR-582 overexpression lead to an enormous increase of apoptotic and dead cells and sensitized the cells against chemotherapeutic agents. The low basal expression of these miRNAs could be increased either by treatment with HDAC-inhibitors (for miR-139) or with DNA-methyltransferase inhibitors (miR-582). To identify putative target genes we performed SILAC followed by mass spectrometry and global gene expression microarrays of miR-582/ miR139 overexpressing cell lines.

Discussion: Here, we show that miRNA-139 and miRNA-582 emerge as tumor suppressor genes, which are downregulated in AML with t(8;21). Both miRNAs reside in host genes of the PDE protein family, suggesting a functional linkage between the two miRNAs.

Friday, June 8th, 2012
11:15 – 12:00 h

9 Pharmacotherapy of malignant diseases – side effects and new approaches

Chair: Prof. Renate Panzer-Grümayer

9.a Influence of tyrosine kinase inhibitors (TKIs) on endocrinological parameters
Ulmer A, Tauer JT, Suttorp M
Dresden

9.b Side effects of tyrosine kinase inhibitors (TKIs) on bone remodelling
Tauer JT, Ulmer A, Hofbauer LC, Suttorp M
Dresden

9.c Preclinical evaluation of a novel treatment strategy to treat high risk ALL
Hasan MN, Queudeville M, Eckhoff SM, Hermann M, Miller S, Trentin L, Debatin KM, Meyer LH
Ulm

9.a

Influence of tyrosine kinase inhibitors (TKIs) on endocrinological parameters

Ulmer A, Tauer JT, Suttorp M

Pediatric Dpt, University Hospital, Dresden, Germany

Background: TKIs - regardless their specifity - exert off-target effects. Currently their influence on endocrine systems - being of particular interest in growing organisms - is not clarified. We investigated growth-related parameters in juvenile rats and pediatric patients (pts) with chronic myeloid leukemia (CML) chronically exposed to TKIs.

Methods: In 29 pts (n= 16 male) and male rats serum testosterone (Testo), inhibin B (IB), and IGFBP3 levels were measured by ELISA. The growing animals were exposed to different TKIs (imatinib, dasatinib, bosutinib) at varying concentrations and time periods.

Results: In rats compared to controls no differences were found for Testo and IB, while IGFBP3 levels were significantly decreased. Also approximately 80% of the pts exhibited Testo and IB levels within age-related reference ranges.

Conclusion: Our data do not confirm a general effect of TKIs on the reproductive system. Clinically individual cases with late onset puberty remain to be investigated. Impairment of longitudinal growth by TKIs has been reported in case studies as well as in animal models. As demonstrated in the rat model influence on the growth hormone axis reasonably mandate to focus on monitoring growth parameters in pts with CML.

9.b

Side effects of tyrosine kinase inhibitors (TKIs) on bone remodelling

Tauer JT, Ulmer A, Hofbauer LC*, Suttorp M

Dpts of Pediatrics & * Intern. Med. III, University Hospital, Dresden, Germany.

Background: Patients with CML on TKI treatment may show altered bone metabolism. We investigated the influence of the TKIs imatinib (IMA), Dasatinib (DASA), and Bosutinib (BOSU) on the growing skeleton in juvenile rats.

Methods: 4 weeks-old rats were chronically exposed to TKIs over a 10 week period. At fixed time points animals were sacrificed to analyze the following parameters: bone length, bone mineral density (BMD), and endocrinological parameters.

Results: Animals treated with IMA and DASA exhibited reduced bone length with reduced trabecular BMD but normal cortical BMD, and altered endocrinological parameters. Intermitted treatment resulted in catch-up growth and partial normalization of bone-specific biochemical markers. DASA revealed additional adverse cardiac effects whereas BOSU showed no pre-eminent side effects at all.

Conclusion: This juvenile rodent model mimicks pediatric chronic TKI exposure with predictable possible side effects. BOSU shows less detectable side effects than other TKI.

9.c

Preclinical evaluation of a novel treatment strategy to treat high risk ALL

Hasan MN, Queudeville M, Eckhoff SM, Hermann M, Miller S, Trentin L, Debatin KM, and Meyer LH

Ulm University, Department of Pediatrics & Adolescent Medicine

We recently showed that rapid engraftment of patient ALL in NOD/SCID mice is indicative for poor patient survival. Moreover, gene expression analysis identified differential expression of molecules regulating the mTOR pathway. We now functionally address mTOR activation assessing P-S6 levels in xenograft ALL and evaluate mTOR inhibition as novel treatment strategy *ex vivo* and *in vivo*.

Increased P-S6 levels were detected in TTL^{short} compared to TTL^{long} xenografts indicating constitutive mTOR hyperactivation in TTL^{short}/early relapse leukemia. Furthermore, the mTOR inhibitors rapamycin and BEZ235 significantly decreased the mTOR activity only in TTL^{short} xenografts. ALL bearing NOD/SCID mice were treated with Rapamycin alone or in combination with multiagent chemotherapy. We observed a significant delay of ALL onset and reduced tumor load in TTL^{short} leukemias upon combination treatment. However the TTL^{long} leukemia showed onset at similar time points with indifferent tumor loads irrespective of treatment modalities.

Thus, mTOR inhibition is a novel therapeutic strategy for the treatment of TTL^{short}/high risk ALL.

Friday, June 8th, 2012
14:00 – 15:45 h

10 Hematology – hematopoiesis and normal blood counts

Chairs: Prof. Karl Welte & Prof. Julia Skokowa

10.a GM-CSF is not able to induce granulopoiesis in CN patients due to absence of LEF-1 transcription factor
Koch C, Welte K, Skokowa J
Hannover

10.b Mechanism of the Elevated UPR in CN Patients but not in CyN Patients Carrying Same ELANE Mutations
Nustede R, Kusnetzova I, Gigina A, Skokowa J, Welte K
Hannover

10.c NAMPT-dependent deacetylation of the hematopoietic-specific lyn-substrate 1 (HCLS1)
Samareh B, Klaus A, Welte K, Skokowa J
Hannover

10.d A role of GADD45b protein in myeloid differentiation and stress response of human hematopoietic cells
Karachunskiy G, Gigina A, Welte K, Skokowa J
Hannover

10.e Acetylation of myeloid-specific transcription factor C/EBP
Kuznetsova I, Welte K, Skokowa J
Hannover

10.f Indirect determination of pediatric blood count reference intervalls
Zierk J, Arzideh F, Haeckel R, Rascher W, Rauh M, Metzler M
Erlangen, Bremen

10.g mRna and Protein Expression Levels of Secretory Leukocyte Protease Inhibitor (SLPI) are Severely Reduced in Patients with Severe Congenital Neutropenia (CN)
Klimenkova O, Ellerbeck W, Gigina A, Skokowa J, Welte K,
Hannover

10.a

GM-CSF is not able to induce granulopoiesis in CN patients due to absence of LEF-1 transcription factor

Koch C, Welte K, Skokowa J

Medical School Hannover, Germany.

G-CSF is successfully used to treat patients with severe congenital neutropenia (CN). However, daily treatment with high doses of G-CSF (up to 50 □g/kg/d) are required to induce granulocyte numbers of above 1000/□l in CN patients. Intriguingly, GM-CSF failed even at high dosages (30 □g/kg/day) to increase the neutrophil numbers, but led to a dramatic increase of eosinophils and monocytes in those patients. We aimed to investigate the mechanisms of abrogated GM-CSF-triggered granulopoiesis in CN patients. Recently, we found that in CN patients “steady-state” granulopoiesis could not be activated due to a lack of LEF-1 and C/EBP□ transcription factors and that G-CSF induces C/EBPß-triggered “emergency” granulocytic differentiation. In the present study, we demonstrated that GM-CSF only slightly induces C/EBPß expression, in comparison to G-CSF. Moreover, in CFU assays, inhibition of LEF-1 in $CD34^+$ hematopoietic cells using LEF-1 shRNA led to completely abolished CFU-G, but unaffected CFU-M production upon stimulation with GM-CSF. Therefore, we concluded, that GM-CSF is not able to induce granulopoiesis in CN patients due to a lack of LEF-1/C/EBP□ and its inability to induce C/EBPß.

10.b

Mechanism of the Elevated UPR in CN Patients but not in CyN Patients Carrying Same ELANE Mutations

Nustede R, Kusnetzova I, Gigina A, Skokowa J, Welte K

Medical School Hannover, Germany.

In severe congenital neutropenia (CN) patients mutated neutrophil elastase (NE) protein induced unfolded protein response (UPR) leading to elevated apoptosis. However, it is unclear, why UPR was not detected in patients with cyclic neutropenia (CyN) carrying the same ELANE mutations. We investigated the mechanism of UPR (activation of ATF6 and ATF6 target genes (GADD34, CHOP and BiP) in CN patients in comparison to CyN patients and to healthy individuals. We detected significantly elevated levels of ATF6 and BiP in myeloid cells of CN patients, in comparison to CyN patients. We transduced the myeloid cell lines HL60 and NB4 with lentiviral constructs contained either wild type (WT) ELANE cDNA, or mutated (MUT) ELANE cDNA and measured expression of ATF6 and ATF6 target genes. We compared the effects of 3 ELANE mutations: C42R, V145-C152del (both presented in CN patients, but not in CyN patients) and S97L (typical for CN and CyN patients) with WT ELANE. In both cell lines C42R MUT, but not V145-C152del MUT or S97L MUT induced expression of ATF6, GADD34, CHOP and BIP, as compared to control transduced cells. In summary, different ELANE mutations have different effects on UPR as judged by ATF6 activation.

10.c

NAMPT-dependent deacetylation of the hematopoietic-specific lyn-substrate 1 (HCLS1)

Samareh B, Klaus A, Welte K, Julia Skokowa J

Medical School Hannover, Germany.

Recently, we demonstrated an essential role of the hematopoietic cell-specific Lyn substrate 1 (HCLS1 or HS1) protein in myeloid differentiation of human and mouse hematopoietic cells. HCLS1 is an interaction partner of HAX1, which is mutated in patients with severe congenital neutropenia (CN). In these patients HCLS1 expression and functions are severely downregulated, leading to “maturation arrest” of myelopoiesis. We also described the new pathway of myeloid differentiation downstream of G-CSF in healthy individuals and in CN patients: G-CSF induced Nampt and NAD^+, which activated NAD+-dependent protein deacetylases, sirtuins. A huge amount of proteins are post-translationally modified by acetylation or deacetylation. However, it is unclear whether changes in the acetylation status activates or suppresses functions of proteins.

In the present study we analyzed if HCLS1 protein could be de-/acetylated and if de-/acetylation of HCLS1 affects its functions during myeloid differentiation. We found that HCLS1 could be acetylated on 4 lysines. We generated rabbit polyclonal antibody specific for each acetylated lysine and found that HCLS1 is acetylated on three lysines in the acute myeloid leukemia cell lines NB4 and HL60 as well as in $CD34^+$ hematopoietic cells. We also found that SIRT1 and SIRT2 interact with HCLS1 protein in NB4 cells. Moreover, specific inhibition of Nampt (FK866) in NB4 cells led to enhanced acetylation of HCLS1 on Lys 123 and 241. Inhibition of SIRT2 (AC93253) elevated HCLS1 acetylation on Lys 123 and 192. Moreover, treatment of $CD34^+$ cells with G-CSF induced interaction between SIRT2 and HCLS1 proteins, migration of SIRT2:HCLS1 complexes and changed acetylation status of HCLS1. Effects of Nampt/SIRT-triggered deacetylation on HCLS1 functions remain to be investigated.

10.d

A role of GADD45b protein in myeloid differentiation and stress response of human hematopoietic cells

Karachunskiy G, Gigina A, Welte K, Skokowa J

Medical School Hannover, Germany.

Growth arrest and DNA-damage-inducible, beta (GADD45b) protein modulates stress responses in mouse myeloid cells. Recently, we demonstrated, that in patients with severe congenital neutropenia (CN) daily treatment with high doses of G-CSF stimulated C/EBPß-dependent emergency stress-induced granulopoiesis.

Based on these findings, we assumed that GADD45b could play a role in the induction of granulopoiesis in CN patients. We assessed GADD45b expression levels in “arrested” promyelocytes of CN patients and surprisingly found severe downregulation of GADD45b expression in CN promyelocytes, in comparison to cyclic neutropenia patients and to G-CSF-treated healthy individuals. Expression of GADD45b target genes (e.g. cyclin B1, p27, CDK6, CDK4, bcl-xl) was also significantly reduced. We further evaluated a possible involvement of GADD45b in myeloid differentiation and stress response of human myeloid cells. GADD45b protein is known to be activated and to migrate into the nucleus after induction of DNA damage (e.g. ultraviolet (UV) irradiation). We found that UV irradiation of the NB4 but not THP1 acute myeloid leukemia cell line induces nuclear GADD45b. Therefore, we further analysed functions of GADD45b in myeloid differentiation and DNA damage in NB4 cells. We found that GADD45b was activated and migrated into the nucleus upon treatment of NB4 cells with ATRA. Inhibition of GADD45b in these cells led to dramatically reduced ATRA-triggered myeloid differentiation, which was in line with diminished expression of myeloid-specific transcription factor C/EBPß, which triggers “stress-induced” myeloid differentiation, but also of C/EBPa, responsible for “steady-state” granulopoiesis. Moreover, transduction of NB4 cells with GADD45b shRNA resulted in significantly enhanced sensitivity to UV irradiation, as documented by elevated apoptosis and reduced proliferation. This was in line with diminished mRNA expression of anti-apoptotic genes bcl-2, mcl-1, survivin as well as elevated mRNA expression of pro-apoptotic gene bad in GADD45b shRNA transduced cells,

as compared to ctrl shRNA transduced cells. p21 mRNA expression was also significantly downregulated after inhibition of GADD45b mRNA.

Taken together, GADD45b plays an important role in myeloid differentiation and stress response of acute myeloid leukemia cell line NB4. Studies of the effects of GADD45b in myeloid differentiation of primary hematopoietic cells in healthy individuals and in CN patients are ongoing.

10.e

ACETYLATION OF MYELOID-SPECIFIC TRANSCRIPTION FACTOR C/EBPα

Kuznetsova I, Welte K, Skokowa J

Medical School Hannover, Germany.

Previously, we described new mechanism of G-CSF-triggered granulocytic differentiation via activation of the enzyme Nicotinamide Phosphorybosyltransferase (Nampt) leading to NAD^+ production and activation of NAD^+-dependent protein deacetylases SIRT1 and SIRT2.

In order to investigate the mechanism of Nampt-triggered myeloid differentiation, we investigated whether myeloid-specific transcription factor C/EBPα could be acetylated. We found that SIRT1 and SIRT2 bind to and activate C/EBPα. We found that C/EBPα is acetylated on Lys 161 and generated rabbit polyclonal antibody specifically recognised acetyl-Lys 161 C/EBPα. We further analysed intracellular localization of acetylated C/EBPα and found that in acute myeloid leukemia cell lines NB4 and HL60 as well as in primary hematopoietic $CD34^+$ cells acetylated C/EBPα was localized in the nucleus. G-CSF treatment of $CD34^+$ cells or ATRA treatment of NB4 cells resulted in the deacetylation of C/EBPα. To evaluate the involvement of Nampt in the deacetylation of C/EBPα we treated NB4 and HL6 cells with the specific inhibitor of Nampt, FK866. We found dramatically elevated levels of acetylated C/EBPα after inhibition of Nampt. Moreover, Nampt and SIRT1 significantly enhanced C/EBPα-triggered activation of reporter gene constructs of C/EBPα target genes G-CSF and G-CSFR and by this inducing myeloid differentiation.

10.f

Indirect determination of pediatric blood count reference intervals

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Determination of pediatric reference intervals (RIs) for laboratory quantities including hematological quantities is complex. The measured quantities vary by age, and obtaining samples from healthy children is difficult. Many available RIs are derived from small sample numbers and split into arbitrary discrete age intervals. To overcome these shortcomings, we applied a refined indirect approach to generate RIs using data derived from 60,000 patient samples of our routine laboratory. Continuous intra-laboratory RIs were determined for blood count quantities from birth to adulthood. Comparison analyses showed that the indirect RIs closely match RIs generated with considerable expense by established methods. The presented approach can easily be transferred to any laboratory quantity and might therefore be used as an alternative method for RI determination where classical approaches cannot be applied.

10.g

mRNA and Protein Expression Levels of Secretory Leukocyte Protease Inhibitor (SLPI) are Severely Reduced in Patients with Severe Congenital Neutropenia (CN)

Klimenkova O, Ellerbeck W, Gigina A, Skokowa J, Welte K

Medical School Hannover, Germany.

Secretory Leukocyte Protease Inhibitor (SLPI) is a cationic serine protease inhibitor with antiprotease, primarily anti-NeutrophilELastase (NE), activities. The molecular interaction and the balance between NE and SLPI is tightly regulated.

We identified severe diminished levels of SLPI mRNA in myeloid cells and in plasma of severe congenital neutropeniapatients (CN), as compared to cyclic neutropeniapatients (CyN) and to healthy individuals. We further analysed whether diminished levels of SLPI could cause „maturation arrest“ of myeloid cells seen in CN patients. We inhibited SLPI in the myeloid cell line NB4 and in hematopoietic $CD34^+$ cells with SLPI-specific shRNA and analysed ATRA- or G-CSF-triggered myeloid differentiation, respectively. Indeed, myeloid differentiation was diminished in NB4 cells and in $CD34^+$ cells transduced with SLPI-specific shRNA, as compared to control shRNAtransduced cells. which was accompanied by G_0/G_1 cell cycle arrest of SLPI shRNAtransduced cells. The mechanisms of “maturation arrest” of granulopoiesis due to SLPI inhibition remain to be investigated.

Friday, June 8th, 2012
16:15 – 17: 45 h

11 Acute leukemias and non-Hodgkin lymphoma – mechanism of disease, treatment response and outcome

Chair: Dr. Markus Metzler

11.a Stress pathways controlling HNSCC dormancy are differentially regulated in pediatric ALL cell lines
Schewe DM
Kiel

11.b Reverse Phase Protein Array (RPPA) of High Risk ALL
Seyfried F, Accordi B, Queudeville M, Eckhoff SM, Milani G, Galla L Giordan M, Kraus J, Basso G, Kestler H, te Kronnie G, Debatin KM, Meyer LH
Ulm, Padova

11.c Role of three childhood acute lymphoblastic leukemia-associated SNPs in a pediatric non-Hodgkin lymphoma cohort
Bartram T, Seidemann K, Burkhardt B, Wössmann W, Zimmermann M, Ellinghaus E, Franke A, Schreiber S, Schrappe M, Reiter A, Stanulla M,
Kiel, Hannover, Muenster, Giessen

11.d Differences in LOH6q and its prognostic impact on pediatric T-LBL and T-ALL
Bonn BR, Rohde M, Zimmermann M, Burkhardt B,
Giessen, Muenster

11.e TGFß signaling in mesenchymal stromal cells and its role in fibrosis associated with down syndrome acute megakaryoblastic leukemia (DS-ML)
Walter C, Reinhardt K, Von Neuhoff N, Reinhardt D, Thakur BK
Hannover

11.f Investigating the role of TGFß signaling in the escape mechanism of leukemic blasts in a myelofibrotic environment
Thakur BK, Hummel O, Fränzel I, Rasche M, Reinhardt K, Reinhardt D,
Hannover

11.a

Stress pathways controlling HNSCC dormancy are differentially regulated in pediatric ALL cell lines

Schewe DM

Allgemeine Pädiatrie UKSH, Kiel, Germany

Introduction: In pediatric ALL, achieving complete remission shortly after therapy initiation isprognostic, but little is known on dormancy of leukemic cells surviving chemotherapy in the bone marrow (BM) niche. InHNSCC, dormancy is maintained by MAPK-dependent regulation of stress pathways in the ER. Mediators of ER-stress orchestrate a cellular response characterized by growth arrest and survival upon chemotherapeutic or microenvironmental stress.

Method: *In vitro* studies to elucidate mechanisms mediating ALL dormancy in the BM microenvironment.

Results: TEL-AML1 positive REH cells display a dormancy signature characterized by high phosphorylation of p38 and eIF2α. SUP-B15 cells (BCR-ABL1 positive) express low levels of these markers. Upon co-culture with MSC cells, grp78 (a p38-regulated ER-chaperone) is upregulated in REH but not in SUP-B15 cells. This is accompanied by growth arrest and marked resistance to L-Asparaginase. Last, only REH cells are able to splice the ER-stress regulated transcription factor XBP-1 upon stress, a response entirely absent in SUP-B15 cells.

Conclusion: p38 and ER-stress pathways may be connected to growth arrest and therapy resistance of TEL-AML1 positive leukemic cells in the BM microenvironment.

11.b

Reverse Phase Protein Array (RPPA) of High Risk ALL

Seyfried F[1], Accordi B[2], Queudeville M[1], Eckhoff SM[1], Milani G[2], Galla L[2], Giordan M[2], Kraus J[1], Basso G[2], Kestler H[1], te Kronnie G[2], Debatin KM[1], Meyer LH[1]

[1]Ulm University, Germany; [2] Padova University, Italy

In a NOD/SCID/huALL model we have recently shown that short time to leukemia (TTL^{short}) of xenografted patient samples determines poor patient outcome. In this study we analyzed 16 xenografted BCP-ALL samples using RPPA and validated the findings by Western Blot.

Comparison of the protein expression data in short vs. long TTL subgroups (shrinkage t-test, $P < .05$, fold change ≥ 1.5) revealed up-regulation of CYCLIN B, ß-CATENIN and ANNEXIN I and down-regulated PKCd in TTL^{short} ALL. Consistently, CYCLIN B is a positive regulator of the cell cycle suggesting an association of cell cycle progression and NOD/SCID engraftment. ß-CATENIN is involved in WNT-signaling and associated with apoptosis inhibition and cell growth. ANNEXIN I is a regulator of inflammation and reported to be over-expressed in hairy cell leukemia. In contrast, down-regulated PKCd has also been reported in poor prognostic T-ALL patients.

Taken together, this study identified differentially expressed proteins in prognostic subgroups of BCP-ALL patients with distinct clinical outcomes, which can be further evaluated as new prognostic markers and therapeutic targets.

11.c

Role of three childhood acute lymphoblastic leukemia-associated SNPs in a pediatric non-Hodgkin lymphoma cohort

Bartram T[1], Seidemann K[2], Burkhardt B[3], Wössmann W[4], Zimmermann M[2], Ellinghaus E[5], Franke A[5], Schreiber S[5], Schrappe M[1], Reiter A[4], Stanulla M[1]

[1]Department of Paediatrics, University Hospital Schleswig-Holstein, Kiel; [2]Department of Pediatric Hematology and Oncology, Hannover Medical School, Hannover; [3]Department of Pediatric Hematology and Oncology, University Children's Hospital Muenster, Muenster; [4]Department of Paediatric Haematology and Oncology, Justus Liebig University, Giessen; [5]Institute of Clinical Molecular Biology, Christian-Albrechts-University, Kiel, Germany.

Aim: Investigation of candidate SNPs previously described to influence the risk of developing childhood acute lymphoblastic leukemia, in pediatric non-Hodgkin lymphoma (NHL).

Methods: Genotyping of SNPs rs4132601 (IKZF1), rs7089424 (ARID5B) and rs2239633 (CEBPE) in 498 German pediatric NHL cases treated on protocol NHL-BFM 95 and 1832 healthy individuals from the PopGen biobank.

Results: No evidence of significant differences regarding the genotype distribution of the three SNPs was found between the entire NHL population as well as specific subgroups and healthy controls.

Conclusion: The analyzed SNPs in IKZF1, ARID5B and CEBPE have no significant influence on developing pediatric NHL.

11.d

Differences in LOH6q and its prognostic impact on pediatric T-LBL and T-ALL

Bonn BR[1], Rohde M[1], Zimmermann M[1], Burkhardt B[1,2]

NHL-BFM study center and Dept. of Ped. Hematology and Oncology in [1]Giessen and [2]Münster, Germany.

Introduction: Although survival rates of pediatric T-cell lymphoma (T-LBL) and related T-cell acute lymphoblastic leukemia (T-ALL) patients are about 70-90%, prognosis in case of relapse is very poor. In contrast to the leukemia type of the disease, little is known about the genetic background of the lymphoma type, and recent analyses reveal certain differences between the two manifestations.

Methods: DNA of 213 T-LBL and 127 pediatric T-ALL cases, treated according to ALL-BFM (type) treatment strategy, was used for loss of heterozygosity (LOH) analyses.

Results: LOH in T-LBL was significantly associated with poor outcome and increased risk of relapse; the common deleted region was mapped to 6q16. In T-ALL, the common deleted region was located in 6q14-15 and not associated with unfavorable prognosis. LOH incidences were similar in both (12% and 13%, respectively).

Conclusion: These results illustrate an important difference between pediatric T-LBL and T-ALL claiming for further analyses concerning this interesting topic. Moreover, it will be very interesting to uncover the underlying alterations in 6q16 responsible for the unfavorable prognosis of these T-LBL patients.

11.e

TGFβ signaling in mesenchymal stromal cells and its role in fibrosis associated with down syndrome acute megakaryoblastic leukemia (DS-ML)

Christiane Walter, Katarina Reinhardt, Nils Von Neuhoff, Dirk Reinhardt and Basant Kumar Thakur

Department of Pediatric Oncology/Hematology, Hannover Medical School, Hannover, Germany

Elevated plasma levels of TGFβ have been shown in children with acute megakaryoblastic leukemia (AMKL). TGFβ is one of the most relevant factors, which induce fibrosis, a common complication associated with AMKL. In children with Down syndrome (DS) but no evidence of leukemia, increased TGFβ level were detected. Because of the relevance of TGFβ signaling in megakaryoblastic leukemia the aim of this study is to analyze the effects of TGFβ on mesenchymal stromal cells (MSC) derived from DS-ML patient (Stroma B) and compare with the healthy control MSC (Stroma A).

We observed that treatment of MSC with TGFβ (10 ng/ml) induced apoptosis (2 fold), which was blocked when TGFβ was neutralized with anti-TGFβ antibody (400 ng/ml). Analysis of the expression level of BAMBI, an inhibitor of TGFβ level signaling, revealed that in Stroma B the expression of BAMBI was higher (>2 fold) in comparison to Stroma A. We observed that treatment with TGFβ increased the mRNA levels of BAMBI in Stroma A and this increase in expression of BAMBI was almost completely blocked when TGFβ was pretreated with anti-TGFβ antibody. In contrary, the mRNA levels of BAMBI in Stoma B remained uneffected after TGFβ treatment. Next, we checked the mRNA levels of CXCL12, ligand of CXCR4, and found that Stroma A expressed relatively higher levels of CXCL12 mRNA compared to Stroma B, where it's levels were almost undetectable. Incubation of either Stroma A or Stroma B with TGFβ induced expression of CXCL12 in both cells, and this induction was partially attenuated when cells were incubated with TGFβ which was pre-blocked with anti- TGFβ antibody.

The major future goal of the current project is to understand the mechanism by which TGFβ regulates the expression of BAMBI and CXCL12 in MSC, and to elucidate its role in altering the migration of leukemic stem cells and induction of fibrosis in cases of DS-ML.

11.f

Investigating the role of TGFβ signaling in the escape mechanism of leukemic blasts in a myelofibrotic environment

Basant Kumar Thakur, Olga Hummel, Imke Fränzel, Mareike Rasche, Katarina Reinhardt and Dirk Reinhardt

Department of Pediatric Oncology/Hematology, Hannover Medical School, Hannover, Germany

Pediatric acute megakaryoblastic leukemia (AMKL) preferentially occurs in infants. The GATA1 mutation associated leukemias affects as a transient leukemia (TL) in newborns with trisomy 21 and as myeloid leukemia of Down syndrome (ML-DS) in infants younger than 5 years of age. Both entities are associated to either liver fibrosis, the most frequent complication of TL within the first weeks or myelofibrosis, which is seen in the majority of cases. Elevated plasma a level of TGFβ has been shown in children with AMKL and has been associated with fibrosis. Here we aimed to identify the mechanism of fibrosis induction associated to a proliferating leukemia.

In primary AMKL, ML-DS and TL-blasts the up-regulation of BAMBI, a transforming growth factor beta (TGFβ) inhibitor, SMAD 6/7 and the moderate down regulation of TGFβ receptors, especially the TBRIII could be found. In-vitro, a dose dependent regulation of these factors by TGFβ is shown in megakaryoblastic cell lines and primary blasts. In addition, TGFβ strongly induced the CXCR4 receptor in blasts and the ligand CXCL12 (SDF-1) on stroma cells derived from patients with AMKL or DS-ML, potentially contributing to an increase bone marrow niche adherence of the leukemic blasts, a typical feature of infant AMKL and ML-DS.

In addition, several other known TGFβ signaling pathway factors and growth factors associated to myelofibrosis were modulated by TGFβ.

In summary, we suggest that in infant megakaryoblastic leukemia, the induction of BAMBI and co-effective SMAD and down regulation of TBRIII contributes to an escape mechanism of leukemic blasts in a myelofibrotic environment. The modulation of the CXCR4-CXCL12 axis further contributes to an increased bone marrow niche adherence.

Friday, June 8th, 2012
18:00 – 19:00 h

12 Special anniversary lectures:
25 Tagungen der Kind-Philipp-Stiftung für Leukämieforschung
Ein persönlicher Rückblick
Prof. Dr. med. Hartmut Kabisch
Universitätsklinikum Hamburg-Eppendorf

G-CSF, a wonderfule molecule
Prof. Dr. med. Karl Welte
Medizinische Hochschule Hannover

Saturday, June 9th, 2012
09:00 – 09:45 h

13 Liver tumors – biology and therapeutic strategies

Chair: Prof. Meinolf Suttorp

13.a The role of the DNMT1/UHRF1/USP7 complex in hepatoblastoma
Trippel F, Joppien S, von Schweinitz D, Längst G, Kappler R,
Munich, Regensburg

13.b The role of the DNMT recruiting protein LSH in hepatoblastoma
Kauffmann D, Trippel F, von Schweinitz D, Längst G, Kappler R,
Munich, Regensburg

13.c Therapeutic potential of CDK-inhibitors in hepatolastoma
Eichenmüller M, von Schweinitz D, Kappler R,
Munich

13.a

The role of the DNMT1/UHRF1/USP7 complex in hepatoblastoma

Trippel F[1], Joppien S[1], von Schweinitz D[2], Längst G[2], Kappler R[1]

[1]Pediatric Surgery, University Munich; [2]Biochemistry III, University Regensburg

Introduction: The cause for epigenetic alterations found in hepatoblastoma (HB) is still unknown. Recently, we identified a trimeric complex consisting of UHRF1 (ubiquitin-like with PHD and ring finger domains 1), DNMT1 (DNA methyltransferase 1), and USP7 (ubiquitin specific peptidase 7) that stimulates both maintenance and *de novo* DNA methylation.

Methods: Knockdown was done by RNA interference, expression measured by real-time PCR and Western blot, methylation analysis by bisulfite sequencing, and DNA binding by ChIP.

Results: UHRF1 binds in concert with DNMT1 and USP7 to promoter regions of tumor suppressor genes relevant in HB cells, namely *HHIP*, *IGFBP3*, and *SFRP1*, which show heavy DNA methylation, enrichment of the repressive H3K27me3 chromatin mark, and gene silencing. Knockdown of *UHRF1* resulted in promoter demethylation and a reduction of H3K27me3, but not reactivation of the genes.

Conclusion: The UHRF1/DNMT1/USP7 complex mediates deep-silencing of tumor suppressor genes in HB.

13.b

The role of the DNMT recruiting protein LSH in hepatoblastoma

Kauffmann D[1], Trippel F[1], von Schweinitz D[2], Längst G[2], Kappler R[1]

[1]Pediatric Surgery, University Munich; [2]Biochemistry III, University Regensburg, Germany.

Introduction: LSH (lympoid specific helicase) is a member of the SNF2 helicase family and plays an essential role in *de novo* methylation of DNA. Hepatoblastoma (HB) displays a high degree of promoter hypermethylation and transcriptional silencing of tumor suppressor genes.

Methods: Knockdown of *LSH* was accomplished by RNA interference, expression measured by real-time PCR and Western blot, methylation analysis by pyrosequencing, and growth by BrdU-assays.

Results: LSH is strongly overexpressed in HB cell lines as well as in most tumor tissues. Knockdown of *LSH* resulted in the reexpression of the silenced tumor suppressor genes *HHIP* and *IGFBP3* and reduced proliferation rates of tumor cells. However, no difference in heavy promoter methylation of both genes was detected.

Conclusion: LSH may play a role in the establishment, but not the maintenance of erroneous methylation patterns. Reactivation of silenced genes, however, indicates an alternative role of LSH in epigenetic regulation.

13.c

Therapeutic potential of CDK-inhibitors in hepatoblastoma

Eichenmüller M[1], von Schweinitz D[1], Kappler R[1]

[1]Pediatric Surgery, University Munich, Germany.

Introduction: The evaluation of new drugs for the effective treatment of high-risk hepatoblastoma (HB) patients is still of utmost importance. Because deregulation of the cell cycle is a hallmark of cancer, we suggest that cyclin-dependent kinase (CDK)-inhibitors might be a therapeutic option.

Methods: Growth inhibition was assessed by MTT-assays, apoptosis by Annexin V staining and Western blot, and gene expression by real-time PCR.

Results: CDKs are frequently overexpressed in HB primary tumors. The CDK-inhibitors Olomoucine and Roscovitine show a dose-dependent cytotoxic effect on the viability of HB cells. Moreover, a massive induction of apoptosis, as evidenced by membrane asymmetry and proteolytic cleavage of caspase 3 and PARP was found. Most interestingly, CDK-inhibitors influenced kinase activity and reduce expression of components of the hedgehog signaling pathway.

Conclusion: The dramatic effects of CDK-inhibitors on growth and important signaling pathways of HB cells recommend these drugs as hopeful new agents for the treatment of HB.

Saturday, June 9th, 2012
10:15 – 11:15 h

14 Brain tumors II – new prognostic markers and therapeutic implications

Chair: Prof. Torsten Pietsch

14.a Analysis of chromosome 1q gain as genetic marker for risk stratification of pediatric ependymoma patients by multiplex ligation-dependent probe amplification (MLPA)
Velez-Char N, Dörner E, zur Mühlen A, von Beuren AO, Rutkowski S, Pietsch T
Bonn, Hamburg

14.b Low-dose Actinomycin-D treatment of ependymomas reactivates p53
Tzaridis TD, Witt H, Pfister SM
Heidelberg

14.c RITA – p53 activation in medulloblastoma as a new approach for treatment?
Gottlieb A, Künkele A,Schramm A, Eggert A, Schulte JH,
Essen

14.d Targeting PLK1 and Aurora kinase in medulloblastoma
Holst M, Westerhout E, Kool M,Caron H, Versteeg R, Clifford S, Rutkowksi S, Pietsch T,
Bonn, Amsterdamm, Newcastle upon Tyne, Hamburg

14.a

Analysis of chromosome 1q gain as genetic marker for risk stratification of pediatric ependymoma patients by multiplex ligation-dependent probe amplification (MLPA)

Velez-Char N[1], Dörner E[1], zur Mühlen A[1], von Beuren AO[2], Rutkowski S[2] & Pietsch T[1]

[1] Dept. Neuropathology, Univ. Bonn;[2] Dept. Pediatr. Oncology, Univ. Med. Center Hamburg, Germany.

Introduction: In retrospective series of patients suffering ependymoma, gain of material of chromosome arm 1q was identified to predict worse outcome. So far, this marker was mainly assessed by FISH analysis.

Methods & Results: To validate this marker in a defined patient cohort, we analysed chromosome 1q in 138 consecutive cases enrolled into the HIT2000 trial in which formalin-fixed, paraffin embedded material was available for DNA extraction. By using MLPA for 5 markers located on chromosome 1q and control markers, we were able to analyse 137/138 cases (99 %) and found 1q gain in 21 cases (15.3 %). Patients with tumors showing 1q gain had a significantly lower 3-year overall survival (OS) (± SE) of 61% ± 13% compared to patients lacking this marker (90% ± 14%, p=0.001). Multivariable analysis demonstrated that 1q gain was an independent risk factor for OS.

Conclusion: We validated chromosomal 1q gain to be a useful genetic marker for risk stratification of pediatric ependymoma patients which can be evaluated by MLPA as a robust, reliable and cost-efficient method.

14.b

Low-dose Actinomycin-D treatment of ependymomas reactivates p53

Tzaridis TD, Witt H, Pfister SM

Department of pediatric Neurooncology, DKFZ, Heidelberg, Germany.

Ependymomas are malignant pediatric brain tumors with frequent p53 inactivation. A *TP53* mutation analysis of 130 primary ependymomas identified a mutation rate of only 3%. Immunohistochemical analysis of 398 ependymomas confirmed previous results correlating the accumulation of non-functional p53 with inferior outcome.

In order to reactivate p53, we evaluated the effects of Actinomycin-D treatment of 2 ependymoma cell lines. The IC-50 of the agent was determined by cell viability assays (0,2-0,7 nM). Subsequenlty, we performed transcriptome analyses of high-dose (100nM), low-dose (5 nM) and non-treated cells showing overexpression of p53 dependent markers (i.e. BBC3) after low-dose treatment. On protein level we validated the Actinomycin-D induced upregulation of p53 interaction partners (MDM-2, p21). Proapoptotic effects of low-dose application of the agent were confirmed by flow cytometry. Thus, Actinomycin-D could comprise a rational therapeutic option for high-risk ependymoma patients, who frequently exhibit p53 inactivation.

14.c

RITA - p53 activation in medulloblastoma as a new approach for treatment?

Gottlieb A, Künkele A, Schramm A, Eggert A, Schulte JH

Department of Pediatric Oncology and Hematology, University Children's Hospital Essen, Germany.

Current therapy of medulloblastoma (MB), the most common malignant brain tumor of childhood, achieves 40-70% survival. Chemotherapy resistence development contributes to treatment failure, where p53 pathway dysfunction plays a key role. Interaction of MDM2 with p53 leads to its degradation, and reactivating p53 functionality using small-molecule inhibitors, such as RITA, to disrupt p53-MDM2 binding may have therapeutic potential. MDM2 expression was analyzed in primary MBs, normal cerebellum and 5 MB cell lines with varying *TP53* mutational status. Cell viability, proliferation and apoptosis and MDM2, p53 and p21 expression were assessed after RITA treatment. Growth and survival of MB xenografts were also assessed after RITA treatment. MDM2 expression was elevated in primary MBs compared to normal cerebellum. All cell lines with wildtype p53 expressed high levels of MDM2. RITA induced apoptosis in 3 of the 5 cell lines, regardless of p53 functional status, and MDM2, p53 and p21 expression increased in all cell lines after RITA treatment. RITA treatment of MB xenografts inhibited tumor growth. RITA decreased cell viability of MB cell lines *in vitro* and in xenografts, independently of the p53 status, and shows promise as a potential therapeutic agent against MB.

14.d

Targeting PLK1 and Aurora kinases in medulloblastoma

Holst M[1],Westerhout E[3], Kool M[3], Caron H[2], Versteeg R[2], Clifford S[4], Rutkowski S[5], Pietsch T[1]

[1]Dept. Neuropathology, University Bonn, [2]Dept. Pediatr., Oncology & [3]Dept.Human Genetics, AMC, Amsterdam, [4]Northern Institute for Cancer Research, Newcastle, [5]Dept. Pediatr. Oncology, Univ. Med. Center Hamburg-Eppendorf, Hamburg, Germany.

Introduction: The Kids Cancer Kinome project is focussing on the human protein kinase family in pediatric tumors to identify and validate novel drug targets. We investigated medulloblastomas as most frequent malignant brain tumor.

Methods: We analysed the expression profiles (Affymetrix microarrays) of medulloblastoma tumor samples and cell lines. Using small molecules inhibitors and lentiviral particles carrying shRNA we examined the effects on cell proliferation (MTS assay), apoptosis (Caspase-glo assay) and cell cycle (flow cytometry).

Results: As promising candidates we identified the overexpressed Plk1 and Aurora kinases which are involved in cell cycle control and are altered in various cancer types. The inhibitors and shRNA caused a decrease of the cell proliferation an increased apoptosis as well as alterations of the cell cycle.

Conclusion: Inhibition of Plk1 or Aurora kinases represents a promising approach for novel targeted strategies in the treatment of medulloblastomas.