

## Open Projects 2009

Please note: this is only a preliminary list

### 1.)

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### Engagement in the MD/PhD program or HBRS:

Supervisor:  Name of student(s):  
Lecturer: ; PhD Kommission: ; No, not yet:

**Animal experiments involved:**  yes  no

### Research focus:

Our research focuses on the genetic susceptibility in asthma and allergies, two of the most common chronic diseases worldwide and a major health concern according to WHO. Asthma and allergies are thought to develop on the basis of genetic susceptibility in combination with environmental factors. We are interested in identifying these genetic factors, the way these genetic variants act and the gene by environment interactions that lead to the disease. We have a long expertise in population genetics based on an existing biobank of 30.000 DNA samples from individuals well phenotyped for asthma and allergy. We use functional genomic approaches, bioinformatic tools and animal models (Drosophila and mouse) very successfully to identify gene function and the role of genetic variants in asthma and allergy.

Recently, we had identified a major susceptibility locus for childhood asthma on chromosome 17q21 in the first genome wide association study on asthma, performed in German and UK samples in 2007. This locus harbors a number of genes that contribute to asthma risk. We now dissect the locus with advanced bioinformatics tools, resequencing and functional genomics using human cell systems as well as transgenic and knockout mice. So far, we had focused on a gene called ORMDL3 of yet unidentified function, which contributes significantly to the chromosome 17q21 asthma susceptibility signal. With this PhD project embedded in one of our BMBF project we extend the analysis to further genes in the locus.

### Specific project and methods applied:

**Title:** The role of genetic variants in the chromosome 17q21 locus in childhood asthma

**Aims:** In the proposed project the role and function of genes and genetic variants from the chromosome 17q21 locus in asthma shall be investigated

**Funding:** BMBF

We have recently identified a locus on chromosome 17q21 as a major susceptibility locus for childhood asthma. These findings were replicated in many populations and studies with exceptional consistency and statistical significance. The locus explains 30% of population attributable risk for asthma in our and a large British population. The susceptibility signal seems to derive from a cluster of genes in the 17q21 locus. One of the target genes in the locus is ORMDL3 and this gene is the focus of a project within the SFB 587. Further genes that contribute to asthma susceptibility are GSDM1, GSDML, ZPBP2 and ZNFN1A3. These genes will be investigated systematically in the PhD project.

**A.** We will apply 2<sup>nd</sup> generation sequencing to sequence all genes in the 17q21 asthma locus to identify potentially causal mutations in these genes. We will use bioinformatic tools to build linkage disequilibrium maps of the region and identify putative functional polymorphisms in these genes. We will genotype newly identified SNPs in the genes in large populations to investigate their specific role on asthma at the population level. That way, we will clarify which genes and which polymorphisms contribute to asthma susceptibility from chromosome 17q21.

**B.** We will use bioinformatic tools to identify the best candidate SNPs for functional studies in these genes. The selected SNPs will be followed up in further in depth experiments using functional genomics tools. In a first step drosophila will be used as a screening tool to identify basic gene expression and function of candidate genes in the respiratory tract. Then, human systems such as promoter assays, DNA- protein and protein- protein interaction will be studied with established tools to investigate the role of polymorphisms in candidate genes, e.g. changes in transcription factor binding, promoter activity, RNA expression and stability, protein confirmation and binding, protein localization and expression. That way, we will clarify how 17q21 polymorphisms contribute to gene function.

**C.** In the final stage, knock out and transgenic mice will be used to investigate the role of the most relevant candidate gene in the locus in asthma and to modulate effects of genetic variation in vivo. (Knock-out and transgene mice will be created by collaboration partners). That way, we will clarify how 17q21 polymorphisms cause asthma.

### **Time schedule**

**1. First year:** The PhD student will focus on population genetics and bioinformatics, learn and perform the following experiments: (A.), Analyzing human association data and drosophila experiments (B.), Preparation of experimental phases in human systems (B.).

**2. Second year:** The PhD student will perform functional genomics in human systems (to study RNA and protein expression, protein interaction analysis, pathway analysis); analyze his/her experimental data and (B.), the PhD student will generate transgenes / knock-out mice carrying the genes of interest for his/her project (C.).

**3. Third year:** The PhD student will perform mouse asthma models (C.), analyze data, write manuscripts; prepare his/her thesis and present project data at international conferences.

### **Group Members:**

Kathrin Suttner, PhD (focus on functional genomics)

Michaela Schedel, PhD (focus on mouse models and functional genomics)

NN, PhD (focus on population genetics, position currently advertised)

Sven Michel, PhD student; 2 PhD student positions currently advertised

XX, MD student; XX, MD student

Sisko Bauer and Michaela Kolletzki, technicians

### **Key References for project**

1. Moffatt\* MF, Kabesch\* M, Liang\* L, (\*firstauthors), Dixon AL, Strachan D, Heath S, Depner M, von Berg A, Bufe A, Rietschel E, Heinzmann A, Simma B, Frischer T, Willis-Owen SA, Wong KC, Illig T, Vogelberg C, Weiland SK, von Mutius E, Abecasis GR, Farrall M, Gut IG, Lathrop GM, Cookson WO. Genetic variants regulating ORM DL3 expression contribute to the risk of childhood asthma. Nature2007 Jul 4.
2. Bouzigon E, Corda E, Aschard H, Dizier MH, Boland A, Bousquet J, Chateigner N, Gormand F, Just J, Le Moual N, Scheinmann P, Siroux V, Vervloet D, Zelenika D, Pin I, Kauffmann F, Lathrop M, Demenais F. Effect of 17q21 variants and smoking exposure in early-onset asthma. The New England journal of medicine2008 Nov 6;359(19):1985-94.
3. Roeder T, Isermann K, Kabesch M. Drosophila in asthma research. Am J Respir Crit Care Med2009;in press.

### **Own references (Originals, limited to 2007-2009):**

1. Suttner K, Depner M, Klopp N, Illig T, Vogelberg C, Adamski J, von Mutius E, Kabesch M. GATA3 polymorphisms are not associated with asthma and atopic phenotypes in German children. **J Allergy Clin Immunol**2009;in press.
2. Suttner K, Ruoss I, Rosenstiel P, Depner M, Pinto LA, Schedel M, Adamski J, Illig T, Schreiber S, von Mutius E, Kabesch M. HLX1 gene variants influence the development of childhood asthma. **J Allergy Clin Immunol**2009 Jan;123(1):82-8 e6.
3. Kormann MS, Ferstl R, Depner M, Klopp N, Spiller S, Illig T, Vogelberg C, von Mutius E, Kirschning CJ, Kabesch M. Rare TLR2 mutations reduce TLR2 receptor function and can increase atopy risk. **Allergy**2009 Feb 12.
4. Weidinger S, O'Sullivan M, Illig T, Baurecht H, Depner M, Rodriguez E, Ruether A, Klopp N, Vogelberg C, Weiland SK, McLean WH, von Mutius E, Irvine AD, Kabesch M. Filaggrin mutations, atopic eczema, hay fever, and asthma in children. **J Allergy Clin Immunol**2008 May;121(5):1203-9 e1.
5. Weidinger S, Gieger C, Rodriguez E, Baurecht H, Mempel M, Klopp N, Gohlke H, Wagenpfeil S, Ollert M, Ring J, Behrendt H, Heinrich J, Novak N, Bieber T, Kramer U, Berdel D, von Berg A, Bauer CP, Herbarth O, Koletzko S, Prokisch H, Mehta D, Meitinger T, Depner M, von Mutius E, Liang L, Moffatt M, Cookson W, Kabesch M, Wichmann HE, Illig T. Genome-wide scan on total serum IgE levels identifies FCER1A as novel susceptibility locus. **PLoS genetics**2008 Aug;4(8):e1000166.
6. Schedel M, Pinto LA, Schaub B, Rosenstiel P, Cherkasov D, Cameron L, Klopp N, Illig T, Vogelberg C, Weiland SK, von Mutius E, Lohoff M, Kabesch M. IRF-1 gene variations influence IgE regulation and atopy. **Am J Respir Crit Care Med**2008 Mar 15;177(6):613-21.
7. Pinto LA, Depner M, Steudemann L, Klopp N, Illig T, von Mutius E, Kabesch M. IL15 gene variants are not associated with asthma and atopy. **Allergy**2008 Dec 24.
8. McLean WH, Palmer CN, Henderson J, Kabesch M, Weidinger S, Irvine AD. Filaggrin variants confer susceptibility to asthma. **J Allergy Clin Immunol**2008 May;121(5):1294-5; author reply 5-6.
9. Kormann MS, Depner M, Hartl D, Klopp N, Illig T, Adamski J, Vogelberg C, Weiland SK, von Mutius E, Kabesch M. Toll-like receptor heterodimer variants protect from childhood asthma. **J Allergy Clin Immunol**2008 Jul;122(1):86-92, e1-8.
10. Koller B, Kappler M, Latzin P, Gaggar A, Schreiner M, Takyar S, Kormann M, Kabesch M, Roos D, Griese M, Hartl D. TLR expression on neutrophils at the pulmonary site of infection: TLR1/TLR2-mediated up-regulation of TLR5 expression in cystic fibrosis lung disease. **J Immunol**2008 Aug 15;181(4):2753-63.
11. Heath SC, Gut IG, Brennan P, McKay JD, Bencko V, Fabianova E, Foretova L, Georges M, Janout V, Kabesch M, Krokan HE, Elvestad MB, Lissowska J, Mates D, Rudnai P, Skorpén F, Schreiber S, Soria JM, Syvanen AC, Meneton P, Hercberg S, Galan P, Szeszenia-Dabrowska N, Zaridze D, Genin E, Cardon LR, Lathrop M. Investigation of the fine structure of European populations with applications to disease association studies. **Eur J Hum Genet**2008 Dec;16(12):1413-29.
12. Pinto LA, Steudemann L, Depner M, Klopp N, Illig T, Weiland SK, von Mutius E, Kabesch M. STAT1 gene variations, IgE regulation and atopy. **Allergy**2007 Dec;62(12):1456-61.
13. Moffatt\* MF, Kabesch\* M, Liang\* L, (\*firstauthors), Dixon AL, Strachan D, Heath S, Depner M, von Berg A, Bufe A, Rietschel E, Heinzmann A, Simma B, Frischer T, Willis-Owen SA, Wong KC, Illig T, Vogelberg C, Weiland SK, von Mutius E, Abecasis GR, Farrall M, Gut IG, Lathrop GM, Cookson WO. Genetic variants regulating ORM DL3 expression contribute to the risk of childhood asthma. **Nature**2007 Jul 4.
14. Kabesch M, Depner M, Dahmen I, Weiland SK, Vogelberg C, Niggemann B, Lau S, Illig T, Klopp N, Wahn U, Reinhardt D, von Mutius E, Nickel R. Polymorphisms in eosinophil pathway genes, asthma and atopy. **Allergy**2007 Apr;62(4):423-8.
15. Depner M, Kormann MS, Klopp N, Illig T, Vogelberg C, Weiland SK, von Mutius E, Combadiere C, Kabesch M. CX3CR1 Polymorphisms Are Associated with Atopy but Not Asthma in German Children. **Int Arch Allergy Immunol**2007 May 15;144(1):91-4.

2.)

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**Engagement in the MD/PhD program or HBRs:**

Supervisor: X Name of student(s): Ratnesh Kumar Srivastav

Lecturer: ; PhD Kommission: ; No, not yet:

**Animal experiments involved:**  yes  no

**Research focus:**

Our research focuses on protein conjugation processes like SUMOylation. More specifically, we are interested in gaining a better understanding how protein SUMOylation is regulated and how SUMOylation regulates cellular and molecular processes.

SUMOylation is protein conjugation process in which the mature SUMO protein is activated by the SAE1/SAE2 SUMO activating protein complex and is then transferred to the E2 conjugating enzyme Ubc9. This can directly or with the help of SUMO ligases transfer the SUMO to lysine residues of a target protein. SUMOylation is involved in different cellular processes and seems to be most important for the regulation of inter- and intramolecular protein-protein interactions. While SUMOylation seems not to be a direct label for protein degradation in recent work it was found out that SUMOylation dependent Ubiquitination is involved in the degradation of proteins.

In the recent past we have analyzed the SUMOylation of components of the MAP-kinase cascades and the JAK/STAT pathway. Therefore we have developed the Ubc9 fusion directed SUMOylation (UFDS) and the Ubc9/substrate dimerisation dependent SUMOylation (USDSDS) systems. With these systems we have identified new SUMOylation substrates and characterized the interplay of protein tyrosine phosphorylation and SUMOylation of STAT proteins. Furthermore we have developed a new system to identify and study in vivo protein/protein interactions in mammalian cells.

**Specific project and methods applied:**

**Title:** Identification of specific substrates of SUMOylated components of the MAP-kinase cascades and their function in signal transduction

**Aims:** In the proposed project specific substrates of SUMOylated components of the MAP-kinase cascades will be identified and their function in the mitogen activated signal transduction will be investigated.

**Funding:**

Recent work on SUMOylation has found out, that SUMOylation is affected by various environmental stresses and that there is an interplay between stress-induced phosphorylation and SUMOylation. We have recently identified several components of the MAP-kinase cascades (MEK1, ERK2, MK2, MK3 and MK5) to be SUMOylated and have started to characterize the kinase SUMOylation with its possible influence on cellular localization, protein stability and activation. SUMOylation is also very important by regulating protein-protein interactions. Very recently it was shown that SUMOylation and SUMO binding motifs act together to build up regulated protein-protein interactions which are for example act in transcriptional repression. Furthermore it was shown, that the SUMO conjugating enzyme Ubc9 conjugated with SUMO is targeted to special SUMOylation substrate proteins with SUMO interacting motifs. We now could show that this specific in vivo targeting of SUMOylated Ubc9 can be mimicked by SUMO-Ubc9 fusion proteins (Srivastav and Niedenthal, unpublished). Here we will try to find out if there is a specific phosphorylation of SUMO interacting motif carrying proteins by SUMOylated kinases. An interplay between phosphorylation and SUMOylation is well known. So there is a phosphorylation dependent SUMOylation described for a number of proteins and we have shown for the STAT1 protein a mutually exclusive SUMOylation of lysine 703 and

phosphorylation of tyrosine 701. So far nothing is known if SUMOylation of kinases can direct these enzymes to a specific subset of phosphorylation substrates, but the numerous proteins which comprise SUMO interacting motifs makes it very probable that there are specific phosphorylation target proteins for SUMOylated kinases.

In this project we will try to identify specific phosphorylation substrates of SUMOylated components of the MAP-kinase cascades. Therefore we will generate SUMO-MAPK-STREP fusion protein expression plasmids, which then will be expressed in stable human cell lines. Using the streptavidin sepharose we will purify these fusion proteins and copurify substrate proteins that bind to the SUMO-kinase fusion proteins and identify these by mass spectrometry. Additionally we will try to identify substrate proteins of SUMO fused kinases in a yeast two hybrid screen. The phosphorylation of the identified substrate proteins then will be characterized for SUMOylation dependence with SUMO-unfused and SUMOylated kinases. Furthermore the SUMO interacting motifs of the new phosphorylation substrate proteins will be characterized by mutation analysis and the function of the new phosphorylation substrate proteins in cellular signaling will be analyzed.

### **Time schedule**

1. In the first year the mammalian expression plasmids for the SUMO-MAPK-STREP fusion proteins and the yeast expression plasmid for the two hybrid screen have to be cloned. When the first expression plasmids are ready the fusion proteins will be expressed in HEK293 cells and SUMO-MAPK-STREP and MAPK-STREP with substrate proteins will be copurified. Bands for binding protein found only with the SUMO-MAKP-STREP fusion protein will be analyzed by massspectrometry. In parallel binding proteins (substrates) of SUMOylated MAP-kinases will be screened by the yeast two hybrid system. 2. In the second year the identification and verification of substrates of SUMOylated MAP-kinases will be continued, the substrates of SUMOylated MAP-kinases will be confirmed in in vitro kinase assays and the characterization of the cellular function of the SUMOylation dependent phosphorylation will be started. 3. In the third year mainly the cellular function of the phosphorylation by SUMOylated MAP-kinases will be analyzed. Which phosphorylation site(s) and SUMO interacting motif(s) are involved in the phosphorylation by SUMOylated MAP-kinases and how regulates this phosphorylation the function, stability and localization of the substrate proteins.

### **Group Members:**

Ratnesh Kumar Srivastav, PhD student; Susan Schwede, PhD student; Alexander Junemann, Diploma student; Malte Klaus, technician

### **Key references and own references (mainly 2007-2009):for project**

Geiss-Friedlander R.and Melchior,F. (2007) Concepts in sumoylation: a decade on. *Nat Rev Mol Cell Biol.*, 8, 947-956. Review.

Jakobs,A., Koehnke,J., Himstedt,F., Funk,M., Korn,B., Gaestel,M. and Niedenthal,R. (2007) Ubc9 fusion-directed SUMOylation (UFDS): a method to analyze function of protein SUMOylation. *Nat. Methods.*, 4, 245-250.

Jakobs,A., Himstedt,F., Funk,M., Korn,B., Gaestel,M. and Niedenthal,R. (2007) Ubc9 fusion-directed SUMOylation identifies constitutive and inducible SUMOylation. *Nucleic. Acids. Res.*, 35, e109.

Zimnik,S., Gaestel,M. and Niedenthal,R. (2009) Mutually exclusive STAT1 modifications identified by Ubc9/substrate dimerization-dependent SUMOylation. *Nucleic. Acids. Res.*

Srivastav,K.R., Zimnik,S., Klaus,M., Gaestel,M. and Niedenthal,R. Monitoring protein-protein-interaction in mammalian cells by Trans-SUMOylation. (submitted)

Knipscheer,P., Flotho,A., Klug,H., Olsen,J.V., van Dijk,W.J., Fish,A., Johnson,E.S., Mann,M., Sixma,T.K. and Pichler,A. (2008) Ubc9 sumoylation regulates SUMO target discrimination. *Mol Cell.*, 31, 371-382.

SUMO under stress.(2008) Tempé D, Piechaczyk M, Bossis G. *Biochem Soc Trans.*, 36, 874-878. Review.

### 3.)

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#### Engagement in the MD/PhD program or HBRS:

Supervisor:  Name of student(s):  
Lecturer: X; PhD Kommission: ; No, not yet:

Animal experiments involved:  yes  no

#### Research focus:

The age-related macular degeneration (AMD) is a clinically heterogeneous, complex multigenic disorder and the major cause of visual impairment in elderly humans. Both, genetic and environmental factors have been identified as susceptibility factors for AMD. The rhesus monkey is a good animal model for human age-related macula drusen formation, the phenotypic hallmark of AMD. Association studies led to the identification of multiple candidate regions for AMD susceptibility in the human genome, including the chromosomes 1, 2, 3, 4, 5, 6, 8, 9, 10, 12, 15, 16, 17, 19, 20 and 22. Recently, it was shown that rhesus monkeys (*Macaca mulatta*) and humans share orthologous susceptibility genes for age-related maculopathy and drusen formation. Our research focuses on the identification of further susceptibility loci for the formation of macular drusen and age-related maculopathies using the rhesus macaque as an animal model.

#### Specific project and methods applied:

**Title:** The rhesus macaque (*Macaca mulatta*) as an animal model for the identification of susceptibility loci for the formation of macular drusen and age-related maculopathies.

**Aims:** The proposed project aimed to clarify whether chromosomal regions homologous to regions harbouring candidate genes for macular degeneration in humans are also associated with the drusen phenotype in rhesus macaques.

#### **Funding:** none

A. We have mapped a susceptibility locus in rhesus macaques to the homologue of human chromosome 6q14-15, which harbours several candidate genes, implicated in human macular degeneration syndromes on functional or positional grounds, namely *RIMI*, *KCNQ5*, *SLC17A5*, *C6orf7*, *MyoVI*, *TTK*, *GABRR1* and *GABRR2*. Sequence analyses of the candidate genes in drusen affected and non affected rhesus macaque genomic DNAs shall be performed to identify risk alleles associated with macular drusen formation in rhesus monkeys.

B. Two other human candidate genes for AMD, the gene encoding complement factor H (*CFH*) on chromosome 1q31 and the apolipoprotein E (*APOE*) gene on chromosome 19q13 shall also be analyzed for a drusen association in rhesus monkeys.

C. AMD in humans and drusen formation in rhesus monkeys also are known to be associated with sequence variations in the *ARMS2* (age-related maculopathy susceptibility 2, also known

as *LOC387715*) and *HTRA1* (high-temperature requirement factor A1) genes on chromosome 10q26. We recently identified a promoter polymorphism in the *HTRA1* gene of rhesus macaques (-558G>T) which shows a significant association with drusen at the genotypic level. Homozygosity for rhesus monkey *HTRA1* SNP -558G>T is associated with the presence of drusen in the analyzed monkey population. The putative effect of the high risk allele of the associated *HTRA1* promoter SNP on *HTRA1* gene expression will be evaluated using the pSEAP luciferase reporter method in 293T human microvascular endothelial cells as reported by Francis et al. (2008). Real-time PCR analysis shall be performed to quantify the expression of the *HTRA1* gene in the retina of monkeys carrying the low- and high risk alleles of the associated *HTRA1* promoter SNP, respectively.

D. In humans Fritsche et al. (2008) identified an AMD associated 23.3 kb risk haplotype, extending from 3.2 kb centromeric of the *ARMS2* gene to parts of intron1 of the *HTRA1* gene. The orthologous region in the rhesus macaque genome will be analyzed for the presence of drusen formation associated SNPs in a cohort of rhesus macaques with and without macular drusen. Putative disease associated risk haplotypes will be functionally characterized (see above).

E. Finally, immunolocalization studies of the ARMS2 and HTRA1 proteins in the retina of rhesus monkeys will be performed, to analyze whether the tissue and cell-specific expression pattern of both protein mimics the human situation.

### **Group Members:**

Britta Marohn, PhD student

Melanie Scholz, Master of Science student

### **Key References for project (mainly 2005-2009)**

Fritsche L.G., Loenhardt T., Janssen A., Fischer S.A., Rivera A., Keilbauer C.N., Weber B.H.F. Age-related macular degeneration is associated with an unstable ARMS2 (LOC387715) mRNA. Nat. Genet. 2008, 40:892-896.

Francis P.J., Appukuttan B., Simmons E., Landauer N., Stoddard J., Hamon S., Ott J., Ferguson B., Klein M., Stout J.T. Neuringer, M. Rhesus monkeys and humans share common susceptibility genes for age-related macular disease. Hum Mol Genet. 2008, 17:2673-2680.

Fisher S.A., Abecasis G.R., Yashar B.M., Zarepari S., Swaroop A., Iyengar S.K., Klein B.E., Klein R., Lee K.E., Majewski J., Schultz D.W., Klein M.L., Seddon J.M., Santangelo S.L., Weeks D.E., Conley Y.P., Mah T.S., Schmidt S., Haines J.L., Pericak-Vance M.A., Gorin M.B., Schulz H.L., Pardi F., Lewis C.M., Weber, B.H. Meta-analysis of genome scans of age-related macular degeneration. Hum Mol Genet. 2005, 14:2257-2264.

### **Own references (mainly 2005-2009):**

Singh K.K., Ristau S., Dawson W.W., Krawczak M., Schmidtke J. Mapping of a macular drusen susceptibility locus in rhesus macaques to the homologue of human chromosome 6q14-15. Exp Eye Res. 2005, 81:401-406.

Singh K.K., Dawson W.W., Krawczak M., Schmidtke, J. IMPG1 gene variation in rhesus macaques macular drusen. Vet Ophthalmol. 2007, 10:274-277.

Singh KK, Krawczak M, Dawson WW, Schmidtke J. Association of HTRA1 and ARMS2 gene variation with drusen formation in rhesus macaques. Exp Eye Res. 2008, 88:479-482.

#### 4.)

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#### Engagement in the MD/PhD program or HBRS:

Supervisor:  (CK) Name of student(s):  
Lecturer:  (CK); PhD Kommission:  (CK); No, not yet:

**Animal experiments involved:**  yes  no

#### Research focus:

Severe congenital neutropenia is an inherited bone marrow failure syndrome characterized by recurrent, severe bacterial and fungal infections due to the lack of mature neutrophils in the periphery. Treatment with recombinant granulocyte stimulating factor (r-GCSF) is effective in most patients at increasing neutrophil counts and decreasing the frequency and severity of infections, nonetheless, patients show impaired functional neutrophil responses and are at a significant risk of developing a myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML).

The genetic defects underlying this disorder remain only partially understood. While a fraction of patients have mutations in the *neutrophil elastase (ELA2)*, *HAX1* or *G6PC3* gene, respectively, approximately 30-40% of SCN patients are of unknown genetic etiology. Our lab aims at elucidating the genetic defects and molecular pathophysiology underlying this disease. Thus, in recent years we have been able to contribute substantially to the field by identifying HAX1 mutation in autosomal-recessive severe congenital neutropenia (Kostmann syndrome) (Klein C et al, Nat Genet 2007). Moreover, we recently identified a syndromic variant of SCN associated with congenital cardiac and urogenital defects, caused by deficiency G6PC3 (Boztug K et al, NEJM 2009). The proposed project aims at identifying a novel genetic cause of SCN in a large, consanguineous family in which mutations in known neutropenia genes have been excluded.

The lab consists of a junior research group led by Kaan Boztug, which is integrated in the Department of Pediatric Hematology and Oncology. The junior group is closely interlinked with the well-established group led by Christoph Klein (head of Department of Pediatric Hematology/Oncology). Taken together, our lab has made significant contributions to the field of Hematology, Immunology and Genetics in recent years.

#### Specific project and methods applied:

**Title:** *Identification and molecular characterization of a novel genetic defect in severe congenital neutropenia*

**Aims:** *Identification of a novel SCN gene and elucidation of the underlying molecular pathophysiology*

**Funding:** *Fritz Thyssen Foundation*

**Significance:** Knowledge of the underlying genetic defects in severe congenital neutropenia is the basis for an enhanced understanding of normal and impaired myelopoiesis in general and the development of severe congenital neutropenia. The molecular pathophysiology of this disease is hitherto largely unknown but will be essential to understand malignant transformation in this disorder as well as enabling the development of more specific therapies such as gene therapy in the future.

## Specific aims and methods:

### **1. Identification of a novel genetic defect underlying severe congenital neutropenia**

We have identified a consanguineous family with several family members affected with severe congenital neutropenia (SCN). DNA samples from these family members are readily available, in addition, we have obtained immortalized EBV-B-cell lines from 2 affected siblings and their parents. SNP-chip base homozygosity mapping has been performed and has yielded a significant linkage interval (LOD score >3), which contains a total of around 30 genes. For this project, we will assess all candidate genes in this interval based on the literature available, and then prioritize which genes should be assessed first. For this purpose, we will perform PCR amplification using efficient primers and subsequent DNA sequencing of the corresponding products. For large genes, extraction of mRNA, and sequencing of the corresponding cDNA may be an alternative. In the past, we have also gained significant experience with more difficult scenarios, e.g. identification of a mutation after microarray-based mRNA expression profiling or detection of large genomic deletions using long range PCR techniques. Thus, we will be able to apply such techniques at any time during the phase of identification of the candidate gene.

### **2. Validation of the candidate gene using reconstitution experiments (functional complementation assays) and detailed characterization of the mutated candidate gene using functional assays such as apoptosis assays, differentiation assays, evaluation of the unfolded protein response etc.**

After identification of a homozygous mutation, we will assess segregation of this mutation amongst other affected and non-affected family members. Studies in primary patient cells including neutrophils, peripheral blood mononuclear cells (PBMCs) and non-hematopoietic cells such as fibroblasts will enable us to perform a variety of cellular and molecular studies to analyze the molecular pathophysiology of this disorder. At present, it is difficult to define such experiments in detail, as the identity of the novel genetic defect is yet unknown. Nonetheless, as other genetic variants are associated with increased apoptosis of myeloid cells, increased activation of the so-called unfolded protein response or destabilization of the mitochondrial membrane potential (as in HAX1-deficiency), we will assess such defects in the patients from this family as well. Furthermore, we will perform transmission electron microscopy (TEM) to evaluate ultrastructural abnormalities associated with the genetic defect. The analyses will help us determine further ensuing assay to be performed.

The functional relevance of the mutation will also be evaluated using complementation assays. For this purpose, appropriate retroviral vectors will be generated. Purified, CD34+ hematopoietic progenitor and stem cells will be transduced and differentiated in vitro into myeloid cells. In these cells, we will show reconstitution of the cellular and molecular phenotype associated with the genetic defect.

Furthermore, depending on the identity of the genetic defect, we may initiate a Yeast-two-hybrid screen in order to identify potential interaction partners. This may be of particular interest in order to understand interaction partners and pathways which are involved in the pathophysiology of this disorder.

### **3. Assessment of frequency of the novel genetic defect in a cohort of patients with severe congenital neutropenia (SCN) of unknown genetic etiology, genotype-phenotype correlation studies**

After identification, we will also initiate genetic studies in a cohort of around 200 patients with severe congenital neutropenia of unknown genetic etiology. These studies will be possible thanks to our close, ongoing collaborations with the Professor Karl Welte and the international SCN registry (SCNIR) based at Hannover Medical School. These studies will be important to assess frequency of the novel genetic defect as well as to establish genotype-phenotype correlations. These studies are essential for a better definition of genetic subgroups, treatment response and clinical phenotype including risk of leukemogenesis in this disorder.

Conclusion: Our studies will lead to a considerable improvement of the genetic and molecular understanding of not only this disorder specifically, but also of the molecular pathophysiology underlying disturbed hematopoiesis in a more general sense. They will also lay ground for definition of clinical subgroups in this disorder as well as the development of future, patient-oriented therapeutic strategies such as hematopoietic stem cell gene therapy.

### **Time schedule**

1. Year 1: Identification of novel genetic defect in SCN
2. Year 2-3: Functional characterization of genetic defect, assessment of frequency of genetic defect, genotype-phenotype studies

### **Group Members:**

Kaan Boztug, MD, postdoctoral fellow, junior research group leader  
Fatih Noyan, PhD, postdoctoral fellow  
Giridharan Appaswamy, PhD, postdoctoral fellow  
Daniel Kotlarz, MD, MD/PhD student  
Inés Avedillo Díez, PhD student  
Anna Gatzke, PhD student  
Dhaarini Murugan, MD/PhD student  
Ankita Minhas, MD/PhD student  
Jana Diestelhorst, technical assistant  
Marie Böhm, technical assistant  
Linda Engling, technical assistant

### **Key References for project**

Bohn G, Allroth A, Brandes G, Thiel J, Rathinam C, Glocker E, Schäffer AA, Teis D, Zeidler C, Geffers R, Buer J, Huber LA, Welte K, Grimbacher B, **Klein C**. A novel human primary immunodeficiency syndrome caused by deficiency of the endosomal adaptor protein MAPBPIP. *Nat Med* 13:38-45, 2007

**Klein C**, Grudzien M, Appaswamy G, Germeshausen M, Sandrock I, Schäffer A.A., Rathinam C., **Boztug K**, Schwitzer B, Rezaei B, Bohn G, Carlsson G, Fadeel B, Dahl N, Palmblad J, Henter JJ, Zeidler C, Grimbacher B, Welte K (2007). Deficiency of HAX1 causes severe congenital neutropenia (Kostmann disease). *Nat Genet* 39:86-92, 2007

**Boztug K**, Appaswamy G, Ashikov A, Schäffer AA, Salzer U, Diestelhorst J, Germeshausen M, Brandes G, Lee-Gossler J, Noyan F, Gatzke AK, Minkov M, Greil J, Kratz C, Petropoulou T, Pellier I, Bellanné-Chantelot C, Rezaei M, Mönckemöller K, Irani-Hakimeh N, Bakker H, Gerardy-Schahn R, Zeidler C, Grimbacher B, Welte K, **Klein C**. A novel syndrome with severe congenital neutropenia caused by mutations in *G6PC3*. *New Engl J Med* 360:32-43, 2009

Schäffer AA, **Klein C**. Genetic Heterogeneity in Severe Congenital Neutropenia: How Many Aberrant Pathways Can Kill a Neutrophil? *Curr Op Allergy Clin Immunol* 7:481-494, 2007

**Boztug K**, Zeidler C, Welte K, **Klein C**. Congenital neutropenia syndromes. *Immunol Allergy Clinics North America* 28:259-75, 2008

### **Own references (2005-2009, selection):**

**Boztug K**, Dewey R, **Klein C**. Development of hematopoietic stem cell gene therapy for Wiskott-Aldrich Syndrome. *Curr Op Mol Therap* 8:390-395, 2006

Rathinam C, Geffers R, Yücel R, Buer J, Welte K, Möröy T, **Klein C**. The transcriptional repressor Gfi1 controls STAT3-dependent dendritic cell development and -function. *Immunity* 22:717-728, 2005

Jung J, Bohn G, Allroth A, **Boztug K**, Brandes G, Sandrock I, Schäffer AA, Schilke R, Welte K, Grimbacher B, **Klein C**. Identification of a genomic deletion in the *AP3B1* gene causing Hermansky-Pudlak syndrome, type 2. *Blood* 108:362-9, 2006

Bohn G, Allroth A, Brandes G, Thiel J, Rathinam C, Glocker E, Schäffer AA, Teis D, Zeidler C, Geffers R, Buer J, Huber LA, Welte K, Grimbacher B, **Klein C**. A novel human primary immunodeficiency syndrome caused by deficiency of the endosomal adaptor protein MAPBPIP. *Nat Med* 13:38-45, 2007

**Klein C**, Grudzien M, Appaswamy G, Germeshausen M, Sandrock I, Schäffer A.A., Rathinam C., **Boztug K**, Schwinzer B, Rezaei B, Bohn G, Carlsson G, Fadeel B, Dahl N, Palmblad J, Henter JI, Zeidler C, Grimbacher B, Welte K (2007). Deficiency of HAX1 causes severe congenital neutropenia (Kostmann disease). *Nat Genet* 39:86-92, 2007

Ju Z, Jiang H, Jaworski M, Rathinam C, Gompf A, **Klein C**, Trumpp A, Rudolph KL. (2007) Telomere dysfunction induces environmental alterations limiting hematopoietic stem cell function and engraftment. *Nat Med* 13:742-7, 2007

Germeshausen M, Grudzien M, Zeidler C, Abdollahpour H, Yetgin S, Rezaei N, Ballmaier M, Grimbacher B, Welte K, **Klein C**. Novel HAX1 mutations in patients with severe congenital neutropenia reveal isoform-dependent genotype-phenotype associations. *Blood* 111:4954-7, 2008

**Boztug K**, Germeshausen M, Avedillo-Diez I, Gulacsy V, Diestelhorst J, Ballmaier M, Marodi L, Welte K, Chernyshova LI, **Klein C**. Multiple independent second-site mutations in two siblings with somatic mosaicism for Wiskott-Aldrich syndrome. *Clin Genet* 74:68-74 2008

**Boztug K**, Appaswamy G, Ashikov A, Schäffer AA, Salzer U, Diestelhorst J, Germeshausen M, Brandes G, Lee-Gossler J, Noyan F, Gatzke AK, Minkov M, Greil J, Kratz C, Petropoulou T, Pellier I, Bellanné-Chantelot C, Rezaei M, Mönckemöller K, Irani-Hakimeh N, Bakker H, Gerardy-Schahn R, Zeidler C, Grimbacher B, Welte K, **Klein C**. A novel syndrome with severe congenital neutropenia caused by mutations in *G6PC3*. *New Engl J Med* 360:32-43, 2009

5.)

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**Engagement in the MD/PhD program or HBRS:**

**Supervisor:** X **Name of student(s):** Kathrin Steinwede

**Lecturer:** X; **PhD Kommission:** No, not yet

**Animal experiments involved:** Yes

### **Project description**

~~Background: *Streptococcus pneumoniae* throws a global threat on human health. Currently available immunization strategies to combat invasive pneumococcal disease (pneumococcal sepsis) include 23-valent polysaccharide vaccines, which mount only partial protection, or 7- or 10-valent conjugate vaccines, which are particularly effective in immunization of young children, while at the same time providing protection against a limited number of pneumococcal strains only. Future alternative vaccination approaches aim at evaluating extra- and/or intracellular *S. pneumoniae* proteins expressed by all pneumococcal serotypes to improve protection against invasive pneumococcal disease, irrespective of the causative pneumococcal strain. We have shown recently for the first time that basophils enhance humoral immune responses to intact pneumococcal (and other) protein antigens, thereby markedly improving survival in a mouse model of lethal pneumococcal sepsis (ref. 10). The current proposal aims at exploiting basophils as new “cellular adjuvant” to enhance primary and secondary immune responses to pneumococcal protein antigens to improve immunization efficacies against *S. pneumoniae*. To improve the overall vaccination efficacy, we plan to combine a primary and/or secondary immunization of mice with extra- versus intracellular pneumococcal protein antigens along with cytokine-based strategies to activate basophils prior to immunization *in vivo*. Subsequently, mice will be challenged with *S. pneumoniae* to evaluate the degree of protection in the absence or presence of a previous basophil activation. In a second experimental approach, we will address the question, whether immunization with intact extra- or intracellular pneumococcal protein antigen of a given serotype also provides cross-protection against other pneumococcal serotypes. In a third experimental approach, we plan to evaluate the effector cell function of basophils in mice during their progression from pneumonia into septic pneumococcal disease. For this approach, mice will be pretreated with antibodies against defined pneumococcal surface proteins followed by infection with highly virulent *S. pneumoniae* to cause sepsis in mice. To evaluate effector cell functions of basophils, we will make use of established protocols to selectively deplete basophils in antibody-pretreated mice as they progress from pneumonia to pneumococcal sepsis.~~

### **Selected group members:**

~~Claudia Plinke, postdoctoral fellow; Kathrin Steinwede, PhD student; Ines Hahn, PhD student, Nadine Ding, PhD student, Christina Brumshagen, PhD student, Stefanie Henken, PhD student; Anna Klaus, technician, Jennifer Bohling, technician, Ann-Kathrin Janzen, technician.~~

## References:

1. Maus UA, Srivastava M, Paton JC, Mack M, Everhart MB, Blackwell TS, Christman JW, Schlondorff D, Seeger W, and Lohmeyer J. Pneumolysin induced lung injury is independent of leukocyte trafficking into the alveolar space. *J Immunol* 173: 1307-1312, 2004.
2. Srivastava M, Jung S, Wilhelm J, Fink L, Buhling F, Welte T, Bohle RM, Seeger W, Lohmeyer J, and Maus UA. The inflammatory versus constitutive trafficking of mononuclear phagocytes into the alveolar space of mice is associated with drastic changes in their gene expression profiles. *J Immunol* 175: 1884-1893, 2005.
3. Srivastava M, Meinders A, Steinwede K, Maus R, Lucke N, Buhling F, Ehlers S, Welte T, Maus UA. Mediator responses and kinetics of mononuclear phagocyte subset recruitment during acute primary and secondary mycobacterial infections in the lung. *Cell. Microbiol.* 9:738-52, 2007.
4. Maus U, Baeki M, Winter C, Srivastava M, Schwarz MK, Rückle T, Paton JC, Briles D, Mack M, Welte T, Maus R, Bohle R, Seeger W, Rommel C, Hirsch E, Lohmeyer J, Preissner KT. Importance of Phosphoinositide 3 kinase  $\gamma$  in the host defense against pneumococcal infection. *Am J Respir Crit Care Med* 175:958-966, 2007.
5. Winter C, Taut K, Srivastava M, Länger F, Mack M, Briles DE, Paton JC, Maus R, Welte T, Gunn MD, and Maus UA. Lung specific overexpression of CCL2 enhances the host defense to *Streptococcus pneumoniae* infection in mice: role of the CCL2-CCR2 axis. *J. Immunol.* 178:5828-5838, 2007.
6. Winter C, Taut K, Länger F, Mack M, Briles DE, Paton JC, Maus R, Srivastava M, Welte T, Maus UA. FMS like tyrosin kinase 3 ligand aggravates the lung inflammatory response to *Streptococcus pneumoniae* infection in mice: Role of dendritic cells. *J. Immunol.* 179:3099-3108, 2007.
7. Taut K, Winter C, Briles DE, Paton JC, Christman JW, Maus R, Baumann R, Welte T, Maus UA. Macrophage turnover kinetics in the lungs of mice infected with *Streptococcus pneumoniae*. *Am. J. Respir. Cell Mol. Biol.* 38:105-113, 2008.
8. Schreiber O, Steinwede K, Maus R, Länger F, Prokein J, Welte T, Gunn MD, Maus UA. Mice overexpressing CC chemokine ligand 2 (CCL2) in their lungs show increased protective immunity to infection with *M. bovis* BCG. *J. Infect Dis.* 198:1044-1054, 2008.
9. Denzel A, Maus UA, Gomez MR, Moll C, Niedermeier M, Winter C, Maus R, Hollingshead S, Briles DE, Kunz-Schughart LA, Talke Y, Mack M. Basophils enhance immunological memory responses. *Nat. Immunol.* 9:733-742, 2008.
10. Winter C, Herbold W, Maus R, Länger F, Briles DE, Paton JC, Welte T, Maus UA. Important role for CCL2 dependent lung mononuclear phagocyte recruitment to inhibit sepsis in mice infected with *Streptococcus pneumoniae*. *J. Immunol.* 182:4931-4937, 2009.

## 6.)

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### Engagement in the MD/PhD program or HBRS:

Supervisor:  Name of student(s):  
Lecturer: ; PhD Kommission: ; No, not yet: X

**Animal experiments involved:** X yes  no

### Research focus:

Our laboratory focuses on understanding the role of the Notch pathway in different gastrointestinal cancers (GI-cancers), especially pancreatic ductal adenocarcinoma (PDAC), hepatocellular carcinoma (HCC) and cholangiocarcinoma (CC). We are interested in improving our knowledge about the basic molecular changes during carcinogenesis of these tumor types, the significance of stem/progenitor cells and are trying to understand the role of Notch in this connection. We hope to gain insights into therapeutic resistance and can develop new approaches to GI cancer therapy.

The Notch signaling pathway has been implicated in the pathogenesis of a number of malignancies. Notch signaling is activated by interaction of Notch ligands with their receptors, promoting a  $\gamma$ -secretase-dependent cleavage of the Notch receptor and release of the Notch intracellular domain (Notch-IC). Notch IC translocates to the nucleus where it serves to activate transcription by binding to the CSL transcription factor and Mastermind-like transcriptional co-activator MAML. This complex activates the transcription of a number of target genes, including members of the Hairy enhancer of split (Hes) family.

We recently used genetically engineered mice and primary cell culture systems to show that the Notch signaling pathway is required for PDAC progression. We targeted the Notch pathway *in vitro* and *in vivo* by using a specific  $\gamma$ -secretase inhibitor (GSI; MRK-003). We were able to show that MRK-003 effectively inhibits tumor progression and offers new therapeutic potential in treatment-refractory GI malignancies.

### Specific project and methods applied:

**Title:** Analysis and treatment of the Notch signaling pathway in hepatocellular carcinoma and cholangiocarcinoma.

**Aims:** In the proposed project, we wish to investigate the role of Notch signaling during murine/human HCC and CC carcinogenesis. We like to monitor the *in vitro* and *in vivo* inhibition of the Notch signaling pathway by application of a  $\gamma$ -secretase inhibitor and siRNAs. We propose to investigate tumor proliferation, migration and apoptosis after inhibition by  $\gamma$ -secretase inhibitor. We will perform GenChip experiments and examine how Notch signaling pathway components affects cancer stem cells.

**Funding:** Deutsche Krebshilfe

We have recently shown that the  $\gamma$ -secretase inhibitor MRK-003 significantly inhibits tumor progression of PDAC. During liver development hepatocytes and cholangiocytes differentiate from biopotential progenitor cells termed hepatoblasts. Mutations in the JAG1 gene, which encodes a ligand for Notch family receptors, cause Alagille syndrome ( a pleiotropic developmental disorder characterized by cholestasis and jaundice caused by intrahepatic bile duct paucity). Mice doubly heterozygous for the Jag1 null allele and a Notch2 hypomorphic allele exhibit developmental abnormalities of the bile duct system. In the newborn mouse liver, Notch2 and Notch3 are expressed in opposing cell populations, suggesting they play different roles in cell fate determination during bile

duct development. Furthermore it was shown that Notch-1, Notch-3, Notch-4 and Jagged-1 were expressed in hepatocellular carcinoma tissues. In primary sclerosing cholangitis (PSC) livers Notch-3 expression appeared to be increased. In addition Notch-1 were up-regulated by cholangiocytes in primary sclerosing cholangitis and cholangiocarcinoma.

Our data and the current literature define a link between the Notch signaling pathway activation in the context of hepatocyte and cholangiocyte carcinogenesis, and provide novel strategies for pharmacologic treatment by using a  $\gamma$ -secretase inhibitor.

*A. Detailed investigation of Notch signaling pathway components during the carcinogenesis of hepatocellular and cholangiocellular carcinoma.* By using Immunohistochemistry, Western Blot and Real Time PCR we will assess the activation state of different Notch signaling pathway components. We are fortunate to have access to human paraffin tissues, cell lines, genetic engineered mice and Xenografts. We plan a correlation of the up- and down regulated Notch signaling pathway components with epidemiological data like tumor grading, age, previous treatments, laboratory parameters, etc. For the in vivo experiments we are using different established mouse models (see below) which develop precursor lesions, HCCs and CCs as well. We will sacrifice mice at certain time points and plan to isolate a panel of cell lines from the precursor lesions and primary tumors.

*B. Modulations of the Notch signaling pathway by using siRNAs and GSI.* Here we like to investigate the in vitro and in vivo treatment effect of specific siRNAs and the  $\gamma$ -secretase inhibitor DAPT (*N*-[*N*-(3,5-difluorophenacetyl)-*L*-alanyl]-*S*-phenylglycine *t*-butyl ester). First, murine and human HCC and CC cell lines shall be treated with different DAPT concentrations or equivalent volume of DMSO. Besides that, we will treat cell lines with specific siRNAs and a combination of GSI and siRNAs. After each period of treatment cell pellets were prepared and snap frozen for future RNA and Protein experiments. Next murine and human HCC and CC cell lines were injected subcutaneously into flanks of SCID mice. For each cell line 3 mice per group will receive daily drug treatments with vehicle or DAPT by using a 3-days on and 4-days off dose schedule. DAPT and vehicle will be applied intraperitoneally. Mice will be euthanized after 6 weeks of treatment. Post treatment the tumor size will be measured and tumor samples will be snap frozen and fixed in formalin. For further in vivo studies 30 mice with the expression of HBs<sup>+/-</sup> and homozygous loss of Trp53<sup>loxP/loxP</sup>, 30 mice with exclusive homozygous loss of Trp53<sup>loxP/loxP</sup> and 30 mice with expression of HBs<sup>+/-</sup>, ALFP<sub>CRE</sub> and homozygous loss of Trp53<sup>loxP/loxP</sup> are going under treatment with DAPT or vehicle. Mice with HBs<sup>+/-</sup> expression homozygous loss of Trp53<sup>loxP/loxP</sup> develop HCCs (85%) and CCs (15%) between 12 and 15 month of life. Mice with ALFP<sub>CRE</sub> and homozygous loss of Trp53<sup>loxP/loxP</sup> develop mixed tumors (HCC/CC). Mice with HBs<sup>+/-</sup>, ALFP<sub>CRE</sub> and homozygous loss of Trp53<sup>loxP/loxP</sup> show a high number of precursor lesions and tumors. For each mouse group 15 animals will receive daily drug treatment with vehicle or DAPT by using a 3-days on and 4-days off dose schedule. Treatment should start with age 32 weeks and will run for 4 month. During therapy mice will be euthanized at different time points. Tumor tissues will be snap frozen and fixed in formalin.

*C. Investigation of tumor proliferation, migration and apoptosis after inhibition by GSI.* To determine the effect of the  $\gamma$ -secretase inhibitor DAPT on cell proliferation we will use a MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). HCC and CC cells will be incubated with various concentrations of DAPT in triplicate in a 96-well plate for 5 to 6 days at 37°C. After incubation cell number will be counted and the cell absorbance of the cell suspension will be measured. Besides that, HCC and CC cell lines will be harvested for agar cloning. In 6 well plates, different cell concentration will be plated and suspended in low melting agarose and medium. Cells will then incubated for 2 weeks at 37°C in a humidified atmosphere and counterstained with p-i violet. Number and size of colonies will be recorded. For assessing the invasive potential of HCC and CC cells Matrigel Invasion Chambers will be used. For the measurement of apoptosis different mice (s.o.) will be injected intraperitoneally with BrdU and analyzed by Immunohistochemistry.

*D. Genexpressionanalysis and evaluation of cancer stem cells after inhibition by GSI.* This part includes Affymetrix GenChip experiments. In order to investigate the role of Notch during the carcinogenesis of HCC and CC we plan to analyze the RNA of treated and untreated Xenograft tumors as well as treated and untreated cell lines. On the other hand cancer stem cell marker CD130<sup>+</sup> and

CD90<sup>+</sup> shall be investigated after treatment with different GSI concentrations. In addition we will isolate Xenograft tumors and prepare single-cell suspensions from the tumor tissues. After collagenase treatment cells will then be incubated with antibodies and sorted for CD130<sup>+</sup> and CD90<sup>+</sup> cells. In the sorted cells we also plan to check the expression of the different Notch signaling pathway components. Finally we will functionally test the ability of the sorted cells to form tumors in a Xenograft model. This will be done by injecting SCID mice with the different cell populations. The Xenograft tumors will be analyzed by Immunohistochemistry for the expression of Notch signaling pathway components.

### **Time schedule for the above mentioned experiments:**

1. year: A, B, C; 2. year: A, B, C; 3. year: A, B, D

### **Group Members:**

Dr. med. Tim Lankisch; MD; Further employees can be expected.

### **Key References for project**

**Plentz RR**, Park JS, Rhim A, Abravanel D, Hezel AF, Sharma SV, Gurumurthy S, Deshpande V, Kenific C, Settleman J, Majumder PK, Stanger BZ, Bardeesy N. Inhibition of Gamma-Secretase activity inhibits tumor progression in a mouse model of pancreatic ductal adenocarcinoma. **Gastroenterology**, **136:1741-1749; 2009.**

### **Own references**

Faca VM, Song KS, Wang H, Zhang Q, Krasnoselsky AL, Newcomb LF, **Plentz RR**, Gurumurthy S, Redston MR, Pitteri SJ, Pereira-Faca SR, Ireton RC, Katayama H, Glukhova V, Phanstiel D, Brenner DE, Anderson MA, Misek D, Scholler N, Urban ND, Barnett MJ, Edelstein C, Goodman GE, Thornquist MD, Mcintosh MW, DePinho RA, Bardeesy N, Hanash SM. A mouse to human search for plasma proteome changes associated with pancreatic tumor development. **PLoS Med** **2008 Juni 10;5 (6): e123.**

**Plentz RR**, Park YN, Lechel A, Kim H, Nellesen F, Langkopf BHE, Wilkens L, Destro A, Fiamengo B, Mann MP, Roncalli M, Rudolph KL. Telomere shortening and inactivation of cell cycle checkpoints characterize human hepatocarcinogenesis. **Hepatology** **2007 April 45(4):968-976.**

**Plentz RR**, Schlegelberger B, Flemming P, Gebel M, Kreipe H, Manns MP, Rudolph KL, Wilkens L. Telomere shortening correlates with increasing aneuploidy of chromosome 8 on a cellular level in primary human hepatocellular carcinoma. **Hepatology**. **2005 September 42 (3): 522-526.**

**Plentz RR**, Tillmann HL, Kubicka S, Bleck JS, Gebel M, Manns MP, Rudolph KL. Hepatocellular carcinoma and octreotide: treatment results in prospectively assigned patients with advanced tumor and cirrhosis stage. **J Gastroenterol Hepatol**. **2005 September 20: 1422-1428.**

**Plentz RR**, Lankisch TO, Bastürk M, Müller CCM, Kirchhoff T, Gebel M, Bleck JS, Kubicka S, Manns MP, Meier PN, Rudolph KL. A Prospective Analysis Of German Patients With Hepatocellular Carcinoma Undergoing Transcatheter Arterial Chemoembolisation With Or Without Prophylactic Antibiotic Therapy. **J Gastroenterol Hepatol**. **2005 Juli 20: 1134-1136.**

Lechel A, Satyanarayana A, Ju Z, **Plentz RR**, Schaetzlein S, Rudolph C, Wilkens L, Wiemann SU, Saretzki G, Malek NP, Manns MP, Buer J, Rudolph KL. The cellular level of telomere dysfunction determines induction of senescence or apoptosis in vivo. **EMBO**. **2005 Februar 18 (6): 1-7.**

**Plentz RR**, Caselitz M, Bleck JS, Gebel M, Flemming P, Kubicka S, Manns MP, Rudolph KL. Hepatocellular telomere shortening correlates with chromosomal instability and the development of human hepatoma. **Hepatology**. **2004 Juli 40 (1): 80-6.**

**Plentz RR**, Wiemann SU, Flemming P, Kreipe H, Meier PN, Kubicka S, Manns MP, Rudolph KL. Critical short telomeres of epithelial cells characterize the adenoma-carcinoma transition of human colorectal cancer. **GUT**. **2003 Sep;52(9):1304-7.**

Wirth T, Zender L, Schulte B, Mundt B, **Plentz R**, Rudolph KL, Manns M, Kubicka S, Kuhnel F. A telomerase-dependent conditionally replicating adenovirus selective treatment of cancer. **Cancer Res**. **2003 Jun 15;63(12):3181-8.**

Omata M, Dan Y, Daniele B, **Plentz R**, Rudolph KL, Manns M, Piratvisuth T, Chen DS, Tateishi R, Chutaputti A. Clinical features, etiology, and survival of hepatocellular carcinoma among different countries. **J Gastroenterol Hepatol**. **2002 Feb;17 Suppl 1:40-49.**

7.)

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**Engagement in the MD/PhD program or HBRS:**

Supervisor: X Name of student(s):  
Lecturer: X; PhD Kommission: X; No, not yet:

**Animal experiments involved:** x yes  no

**Research focus:**

Our research focuses on the regulation of the IFN response after infection and its consequence on the cellular and systemic antiviral response. The IFN system is the first line of defense against infection. It inhibits viral replication, viral spread and links the innate and adaptive immune response. We are interested in the molecular mechanisms which lead to the induction of IFNs, the quality and quantity of IFN response, and the spatio and temporal induction of IFN and interferon stimulated genes after viral infection.

The induction of IFNs is regulated by members of the Interferon Regulatory Factor (IRF) family. Different family members are responsible for the induction of early and late IFNs. The functions of IFNs are mediated by the induction of ISGs.

Viruses have developed multiple strategies to inhibit or circumvent the induction of IFNs. We recently identified a new IFN-independent antiviral mechanism which leads to an antiviral response in the absence of IFN induction.

**Specific project and methods applied:**

**Title: Inflammation versus infection induced antiviral response through IRF-1**

**Funding:**

IRF-1 is induced by interferons (IFNs) and by all inflammatory events through pro-inflammatory cytokines. IRF-1 is a transcription factor that mediates the transcriptional activation of genes that are induced by IFNs ( so-called ISGs) but also of IFN- $\beta$ . In this way elevated expression of this transcription factor can not only induce an antiviral status through the activation of ISGs, but also lead to an extended expression of ISGs though IFN- $\beta$  induction (feed-forward loop.) We have recently confirmed, that IRF-1 is essential for an IFN-independent antiviral effect. Induction of IRF-1 in cells that cannot react on IFNs raised the expression of ISGs, including viperin, a newly detected antiviral protein. Further experiments indicated that IRF-1 KO mice have a higher susceptibility to virus infection than wt mice.

However, IRF-1 is also responsible for the induction of immune-modulatory activities. These include the activation of all genes acting on antigen processing and presentation, as well as the induction of iNOS. Indeed, experimental work with tumor models shows that IRF-1 has a strong anti-tumor effect that is mediated by the adaptive immune system. Thus, infection as well as inflammation should cause antiviral as well as immune-stimulatory effects through the induction of IRF-1. This hypothesis forms the basis for the PhD work.

A. Investigation of antiviral and immune-modulatory effects in cells that lack the IFN-Receptor (IFNAR-/-). Inflammatory events as well as infections will be tested for their activity in antigen presentation, the activation of ISGs and antiviral effects. After having proven the effects induced by

pro-inflammatory cytokines in cell culture, corresponding experiments will be carried out with relevant inflammation models in IFNAR<sup>-/-</sup> mice.

B. Viruses encode products that have anti-IFN activities. In order to define the potency of the IFN-independent antiviral effect mediated by IRF-1 we will compare the antiviral effects of 1. viruses which inhibit the IFN response or 2. virus mutants, that have deletions in these genes (VSV:AV2; NDV; Influenza: Influenza $\Delta$ NS1). These experiments will be carried out in w.t. mice, IRF-1<sup>-/-</sup> mice, IFNAR<sup>-/-</sup> mice and DKO mice (these are currently created). A comparison of viral replication, viral spread and induction of immune responses will help to understand the impact of IRF-1 in pathogen defense.

### **Time schedule**

1 year: Expression analysis and functional consequences of IRF-1 in different KO cells after infection.

2 year and 3 year: In vivo work: Expression analysis and functional consequences of IRF-1 in w.t. and different KO mice after infection.

### **Group Members (Andrea Kröger Lab):**

*2 PhD students*

*1 Diploma student;*

*1 technician*

### **Key References for project**

Jiang D, Guo H, Xu C, Chang J, Gu B, Wang L, Block TM, Guo JT. Identification of three interferon-inducible cellular enzymes that inhibit the replication of hepatitis C virus. *J Virol.* 2008 82(4):1665-78.

Wang X, Hinson ER, Cresswell P. The interferon-inducible protein viperin inhibits influenza virus release by perturbing lipid rafts. *Cell Host Microbe.* 2007 2(2):96-105.

Severa M, Coccia EM, Fitzgerald KA. Toll-like receptor-dependent and -independent viperin gene expression and counter-regulation by PRDI-binding factor-1/BLIMP1. *J Biol Chem.* 2006 281(36):26188-95.

Helbig KJ, Lau DT, Semendric L, Harley HA, Beard MR. Analysis of ISG expression in chronic hepatitis C identifies viperin as a potential antiviral effector. *Hepatology.* 2005 42(3):702-10.

Hwang S, Kim KS, Flano E, Wu TT, Tong LM, Park AN, Song MJ, Sanchez DJ, O'Connell RM, Cheng G, Sun R. Conserved herpesviral kinase promotes viral persistence by inhibiting the IRF-3-mediated type I interferon response. *Cell Host Microbe.* 2009 5(2):166-78.

### **Own references**

**Kröger A**, Stirnweiss A, Pulverer JE, Klages K, Grashoff M, Reimann J, Hauser H. Tumor suppression by IFN regulatory factor-1 is mediated by transcriptional down-regulation of cyclin D1. *Cancer Res.* 2007 67(7):2972-81.

Ramsauer K, Farlik M, Zupkovitz G, Seiser C, **Kröger A**, Hauser H, Decker T. Distinct modes of action applied by transcription factors STAT1 and IRF1 to initiate transcription of the IFN-gamma-inducible gbp2 gene. *Proc Natl Acad Sci U S A.* 2007 104(8):2849-54

Schirmbeck R, Riedl P, Kupferschmitt M, Wegenka U, Hauser H, Rice J, **Kröger A**, Reimann J. Priming protective CD8 T cell immunity by DNA vaccines encoding chimeric, stress protein-capturing tumor-associated antigen. *J Immunol.* 2006 177(3):1534-42.

Froese N, Schwarzer M, Niedick I, Frischmann U, Köster M, **Kröger A**, Mueller PP, Nourbakhsh M, Pasche B, Reimann J, Staeheli P, Hauser H. Innate immune responses in NF-kappaB-repressing factor-deficient mice. *Mol Cell Biol.* 2006 26(1):293-302.

Stirnweiss A, Klages K, Hauser H, **Kröger A**. Virus induced IRF-1 mediates interferon-independent antiviral effects through vig-1. submitted

## 8.)

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Interested applicants are encouraged to contact me any time.

### **Engagement in the MD/PhD program or HBRS:**

Supervisor: X      Name of student(s): Volker Rust

Lecturer: X;    PhD Kommission: ;    No, not yet: X

**Animal experiments involved:**     yes                    X no

### ***Introduction***

An estimated 170 million people are chronically infected with hepatitis C virus (HCV) worldwide. Approximately 20 % of chronically infected patients will develop liver cirrhosis. Once cirrhosis is established, the rate to develop hepatocellular carcinoma (HCC) is 1-5 % per year. At present, therapeutic options are limited. A vaccine is not available. Thus, there is an urgent need to develop more effective and well-tolerated therapies for hepatitis C. Indeed, a series of newly developed small molecular protease and polymerase inhibitors are in clinical trials right now. However, for those inhibitors viral escape mutations are quickly developing. Interactions between host and viral proteins have a high potential as alternative drug targets since viral escape mutations will also abrogate the essential interaction. Therefore, we have recently focused our work on the identification of viral and host factors which are required for virus replication and virion formation.

### ***Research focus***

In previous work, we and others could show that HCV replicates its genome in a membrane-associated replication complex, composed of viral proteins, replicating RNA, and altered cellular membranes (reviewed in Moradpour et al., 2007, see also Wölk et al., 2000, Ivashkina et al., 2002, Egger et al., 2002, Wölk et al., 2008). We have identified specific membrane alterations, termed 'membranous webs', as sites of HCV RNA replication (Egger et al., 2002). We also established a live cell imaging system to analyze functional HCV replication complexes in host cells. In our studies, we could identify two modes of HCV replication complex trafficking which reflect physiologic ER motility and an ER independent microtubule based saltatory transport mechanism (Wölk et al., 2008). However, the cellular and viral factors involved in replication complex trafficking and membranous web formation remain unknown. With the advent of an infectious cell culture system for HCV (Lindenbach et al., 2005) the complete viral life cycle can be studied (Evans et al, 2007) and the determinants for virion assembly can now also be elucidated.

### ***Specific project and methods applied***

**Title:** Characterization of hepatitis C virus nonstructural protein interaction domains required for the formation and correct subcellular targeting of the viral replication complex.

**Aims:** In this project we want to identify protein domains within the HCV nonstructural proteins NS3, NS4A, NS4B, NS5A, and NS5B which are essential for the colocalization and interaction of these proteins in the viral replication complex. To identify, fine map, and characterize these domains, we will delete and mutate interesting domains these nonstructural proteins and analyze the potential of these mutants to form replication complexes and support viral replication as well as infection. In a collaboration with the Institute for Biomedical Ageing Research of the Austrian Academy of Sciences in Innsbruck, Austria, we are currently planning a screen for peptides which can specifically block these interactions and may guide future drug development. Potential hits will be characterized in our lab for their ability to abrogate the protein interactions observed.

**Funding:** Institutional funding / MHH funding

**Work program/Time schedule:** The project comprises the following work packages:

- a) Based on our own work (Wölk et al., 2000; Ivashkina et al. 2002, Kolli & Wölk, in preparation) and based on the literature we will construct an initial set of HCV nonstructural protein mutants, in which broad protein domains will be deleted or replaced (such as the membrane anchors or the cytoplasmic domains of NS4A, NS4B, NS5A, and NS5B, the protease or helicase domain within NS3, domains I, II, or III of NS5A).
- b) We will then assess the subcellular localization pattern of these mutant constructs by fluorescent microscopy and potentially by cell fractionation assays, and we will compare these with the patterns of the wild type (full length) versions of the proteins.
- c) To further define alterations in protein targeting we will coexpress all mutant constructs with their wild type counterparts. We have previously used this technique successfully to identify regions within one of the nonstructural proteins important for protein-protein interaction (Kolli & Wölk, in preparation). Alterations in subcellular localization will be further characterized by colocalization studies with compartment specific cellular marker proteins.
- d) We have previously shown that all nonstructural proteins colocalize and that one of them is sufficient to target each remaining nonstructural protein into the replication complex compartment (Wölk et al., 2008; Kolli & Wölk, in preparation). Thus, we will determine the capacity of our mutant constructs to show the same distinct colocalization pattern compared to wild type. The aim of these experiments will be to identify protein domains which target the nonstructural proteins into the same subcellular membrane compartment and are essential for the establishment of functional replication complexes.
- e) We will then confirm the deletion construct data by introducing point mutations in the identified domains to identify amino acid residues which are essential for replication complex formation. Interesting point mutants will be introduced in the replicon system or in the infectious cell culture system to further characterize these effects on viral replication.
- f) Optional. In a collaborative screening project with the Institute for Biomedical Ageing Research of the Austrian Academy of Sciences in Innsbruck, Austria, we hope to identify aptameres which specifically block HCV protein-protein interactions. Depending on the interest and time of the PhD student we may be characterized positive hits with the tools established within this project to define the mode of action of the identified aptameres.

**Methods:** Our lab offers the opportunity to learn or deepen ones experience for the following methods: Standard methods of molecular biology. Generation of fluorescently tagged proteins. Study of cell biology and reverse genetics in the HCV replicon and in the infectious HCV cell culture system. Live cell imaging analyses of viral and cellular structures. Fluorescence recovery after photobleaching (FRAP) and fluorescence loss in photobleaching

(FLIP) analyses to study protein diffusion and viral transport processes. High resolution confocal microscopy and deconvolution techniques to study colocalization. Colocalization and comigration analyses in live cells. Computer based image analyses to quantify morphologic and motility parameters.

### **Group members:**

(1) Rajesh Kolli, PhD student, (2) Birgit Ritter, technician, (3) Volker Rust, PhD student.

Our group is located in the Department of Virology with close interactions and joint seminars with about 40 scientists within the institute. We have joined weekly lab meetings with a friendly HCV group (approx. 16 participants).

### **Key references for project and selected own references:**

- [References marked with ●● are good starting points for further readings.]
- Egger D, Wölk B, Gosert R, Bianchi L, Blum HE, Moradpour D, Bienz K. Expression of hepatitis C virus proteins induces distinct membrane alterations including a candidate viral replication complex. *J Virol* 2002;76:5974-5984. (●●□Original identification of membranous web.)
- Engler, OB, Schwendener RA, Dai WJ, Wölk B, Pichler W, Moradpour D, Brunner T, Cerny A (2004). A liposomal peptide vaccine inducing CD8(+) T cells in HLA-A2.1 transgenic mice, which recognise human cells encoding hepatitis C virus (HCV) proteins. *Vaccine* 2004;23: 58-68.
- Evans MJ, von Hahn T, Tscherne DM, Syder AJ, Panis M, Wölk B, Hatzioannou T, McKeating JA, Bieniasz PD, Rice CM Claudin-1 is a hepatitis C Virus co-receptor required for a late step in entry. *Nature* 2007;446:801-805.
- Gosert, R., D. Egger, V. Lohmann, R. Bartenschlager, H. E. Blum, K. Bienz, and D. Moradpour. Identification of the hepatitis C virus RNA replication complex in Huh-7 cells harboring subgenomic replicons. *J Virol* 2003;77:5487-5492.
- Gremion C, Grabscheid B, Wölk B, Moradpour D, Reichen J, Pichler W, Cerny A. Cytotoxic T lymphocytes derived from patients with chronic hepatitis C virus infection kill bystander cells via Fas-FasL interaction. *J Virol* 2004;78: 2152-7.
- Ivashkina N, Wölk B, Lohmann V, Bartenschlager R, Blum HE, Penin F, Moradpour D. The hepatitis C virus RNA-dependent RNA polymerase membrane insertion sequence is a transmembrane segment. *J Virol* 2002;76:13088-13093.
- Lindenbach BD, Evans MJ, Syder AJ, Wölk B, Tellinghuisen TL, Liu CC, Maruyama T, et al. Complete replication of hepatitis C virus in cell culture. *Science* 2005;309:623-626. (●●□Characterization of the first infectious cell culture system for HCV.)
- Moradpour D., Penin D, Rice CM. Replication of hepatitis C virus. *Nat. Rev. Microbiol.* 2007;5:453-463. (●●□Review on hepatitis C virus molecular virology.)
- Wölk B, Büchele B, Blum HE, Moradpour D, Rice CM. A dynamic view of hepatitis C virus replication complexes. *J Virol* 2008;82:10519-31. (●●HCV replication complex motility.)
- Wölk B, Gremion C, Ivashkina N, Engler OB, Grabscheid B, Bieck E, Blum HE, et al. Stable human lymphoblastoid cell lines constitutively expressing hepatitis C virus proteins. *J Gen Virol* 2005;86:1737-1746.
- Wölk B, Sansonno D, Kräusslich HG, Dammacco F, Rice CM, Blum HE, Moradpour D. Subcellular localization, stability, and trans-cleavage competence of the hepatitis C virus NS3-NS4A complex expressed in tetracycline-regulated cell lines. *J Virol* 2000;74:2293-2304. (●●□Characterization of the NS3/4B membrane anchor.)

## 9.)

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### Engagement in the MD/PhD program or HBRs:

Supervisor:  Name of student(s):  
Lecturer: ; PhD Kommission: ; No, not yet: x

**Animal experiments involved:**  yes  no

### Research focus:

Our research focuses on the ubiquitin-dependent regulatory mechanisms involved in signal transduction via interleukin-1 receptor (IL-1RI) and Toll-like receptors (TLRs). This is an exciting novel signalling mechanism and the signal transduction pathways triggered by IL1R/TLRs turned out to be a paradigm for the interplay between phosphorylation and ubiquitination.

Signalling through IL1R/TLRs is pivotal for pathogen detection and clearance by the innate immune system. IL-1RI/TLR signalling is regulated by protein kinases of the Interleukin-1 receptor associated kinase (IRAK) family. We recently demonstrated the modification of IRAK1 with an unusual polyubiquitin chain that is linked via Lysine 63 (K63) of ubiquitin, which is key for the IL-1-dependent activation of the transcription factor nuclear factor  $\kappa$ B (NF $\kappa$ B). IRAK2 has been shown to be important for IL-1RI and TLR signalling as well. Studies using knockout mice indicate that IRAK1 and IRAK2 have redundant and non-redundant functions. The regulatory mechanisms and molecular details of IRAK1- and IRAK2-dependent signalling remain to be identified. In our future research we are going to address this aspect of IL-1RI/TLR signalling.

### Specific project and methods applied:

#### Title: Spatial and Temporal Organization of Interleukin-1 Receptor Signalling

**Aims:** In the proposed project the spatial and temporal organization of signalling via the interleukin-1 receptor shall be investigated with a special focus on the roles of the Interleukin-1 receptor associated kinases IRAK1 and IRAK2.

**Funding:** State Funds (Lower Saxony)

Interleukin 1 (IL-1) has been reported to stimulate the polyubiquitination and disappearance of IRAK1 within minutes. It has been thought that the polyubiquitin chains attached to IRAK1 are linked via Lys48 of ubiquitin leading to its destruction by the proteasome, and explaining the rapid IL-1-induced disappearance of IRAK1. We demonstrated recently, that IL-1 stimulates the formation of K63-pUb-IRAK1 and not K48-pUb-IRAK1.

Unexpected recent discoveries suggest a novel regulatory role for IRAK1 degradation. IL-1RI/TLR-mediated signal transduction appears to be divided into an early IRAK1-dependent phase and a late IRAK2-dependent phase. We postulate that the IRAK1 degradation is a key regulatory mechanism for the transition from the early to the late phase.

- A. To test this hypothesis we will identify and inhibit the machinery for IRAK1 degradation. The molecular mechanisms of the IRAK2-dependent signal transduction are completely unknown. We will identify interaction partners and potential kinase substrates of IRAK2.
- B. Since IRAK1 polyubiquitination is crucial of IL-1RI/TLR signalling, it is reasonable to assume that IRAK2 is polyubiquitinated as well after receptor stimulation. Thus, we will investigate whether is the case and how this is integrated in the IRAK2-dependent signal transduction.

- C. Since both IL-1RI and TLR4 are internalized upon ligand binding, we hypothesize that the IRAK1-dependent early phase signalling takes place at the plasma membrane, whereas the IRAK2-dependent signalling events are initiated in the late phase from an intracellular compartment. To test this hypothesis, we will analyse the intracellular localization and trafficking of IRAK1 and IRAK2.

### **Time schedule**

- 1<sup>st</sup> Year: Identification of inhibitors of IRAK1 degradation; analysis of inhibitor effects on signal transduction; identification of IRAK1/2 interacting proteins; analysis of IRAK2 ubiquitination, identification of lysine residues involved and topology of polyubiquitin chains; studies into the intracellular localization of IRAK1/2 by confocal microscopy, cloning of GFP-tagged IRAK1/2 for live-cell-imaging, establishment of stable-cell lines expressing GFP-tagged IRAK1 and IRAK2 using immortalized IRAK1- or IRAK2-deficient mouse embryonic fibroblasts.
- 2<sup>nd</sup> Year: Cloning of IRAK2 mutants, deletion and individual mutants, e.g. lysine mutants that cannot be ubiquitinated, establishment of stable-cell lines expressing IRAK2 wild type and mutant forms either untagged, with a small tag or GFP-tagged using immortalized IRAK2-deficient mouse embryonic fibroblasts.
- 3<sup>rd</sup> Year: Analysis of stable-cell lines with regard to defects in signal transduction, protein-protein interactions and intracellular localization of IRAK2.

### **Group Members:**

Dr. Mark Windheim, Group Leader;

### **Key References for project**

Conze, D. B., Wu, C. J., Thomas, J. A., Landstrom, A., and Ashwell, J. D. (2008). Lys (K)63-linked polyubiquitination of IRAK-1 is required for IL-1 receptor- and Toll-like receptor-mediated NF- $\kappa$ B activation. *Mol. Cell. Biol.* 28, 3538-47.

Kawagoe, T., Sato, S., Matsushita, K., Kato, H., Matsui, K., Kumagai, Y., Saitoh, T., Kawai, T., Takeuchi, O., and Akira, S. (2008). Sequential control of Toll-like receptor-dependent responses by IRAK1 and IRAK2. *Nat. Immunol.* 9: 684-691

Ordureau, A., Smith, H., Windheim, M., Peggie, M., Carrick, E., Morrice, N., Cohen, P. (2008) The IRAK-catalysed activation of the E3 ligase function of Pellino isoforms induces the Lys63-linked polyubiquitination of IRAK1. *Biochem. J.* 409: 43-52

Windheim, M., Stafford, M., Peggie, M., and Cohen, P. (2008) Interleukin-1 (IL-1) induces the Lys63-linked polyubiquitination of IL-1 receptor-associated kinase 1 to facilitate NEMO binding and the activation of I $\kappa$ B kinase. *Mol. Cell. Biol.* 28: 1783-1791

### **Own references:**

Ordureau, A., Smith, H., Windheim, M., Peggie, M., Carrick, E., Morrice, N., Cohen, P. (2008) The IRAK-catalysed activation of the E3 ligase function of Pellino isoforms induces the Lys63-linked polyubiquitination of IRAK1. *Biochem. J.* 409: 43-52

Windheim, M., Stafford, M., Peggie, M., and Cohen, P. (2008) Interleukin-1 (IL-1) induces the Lys63-linked polyubiquitination of IL-1 receptor-associated kinase 1 to facilitate NEMO binding and the activation of I $\kappa$ B kinase. *Mol. Cell. Biol.* 28: 1783-1791

Windheim, M., Peggie, M., and Cohen, P. (2008) Two different classes of E2 ubiquitin-conjugating enzymes are required for the mono-ubiquitination of proteins and elongation by polyubiquitin chains with a specific topology. *Biochem. J.* 409: 723-729

Windheim, M., Lang, C., Peggie, M., Plater, L.A., and Cohen, P. (2007) Molecular mechanisms involved in the regulation of cytokine production by muramyl dipeptide. *Biochem. J.* 404: 179-190

Zhang, M., Windheim, M., Roe, M.S., Peggie, M., Cohen, P., Prodromou, C., and Pearl, L.H. (2005) Chaperoned ubiquitylation- crystal structures of the CHIP U box E3 ubiquitin ligase and a CHIP-Ubc13-Uev1a complex. *Mol. Cell* 20: 525-538

10.)

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**Engagement in the MD/PhD program or HBRs:**

Supervisor: X      name of student(s): Dirk Heckl

Lecturer: ; PhD Kommission: ; No, not yet:

**Animal experiments involved:**      X yes                       no

**Research focus:**

Hematopoietic stem cell (HSC) gene therapy using retroviral vectors has shown remarkable success for the treatment of monogenic diseases such as severe combined immunodeficiency diseases (SCID) or chronic granulomatous disease (CGD). However, hematopoietic clonal disorders induced by vector mediated insertional mutagenesis such as myelodysplasias or acute leukemias have been observed, prompting major efforts to better define and predict these genotoxic risks. Most of these studies have been performed in murine model systems. Sole use of murine models may be problematic, however, as murine and human hematopoiesis clearly display differences. Mice have a significantly higher daily hematopoietic cell turnover including a much faster replication rate of HSCs and in general rodent as compared to human cells display a markedly higher transformation potential. Therefore, complimentary studies with primary human cells appear necessary.

As clinical success of HSC transplantation as well as HSC gene therapy depends on the isolation of sufficient numbers of donor stem cells, in certain situations in vitro amplification of HSCs prior to use has drawn considerable interest. Here, recently a new set of growth factors has been introduced that enable massive expansion of human HSC. However, in the context of HSC gene therapy in vitro expansion strategies pose several potential problems. Thus insertional events promoting proliferation may induce clonal selection and/or malignant transformation already in vitro and preleukemic or leukemic cells could amplify even before transplantation. In addition, the longterm repopulation potential of HSCs and their contribution to the different hematopoietic lineages may be hampered. (246/250 words)

**Specific project and methods applied:**

**Title:** Humanized models to assess the genotoxicity of viral vectors in the context of hematopoietic stem cell expansion

**Aims:** The aim of this project is to establish and to evaluate model systems for the assessment of genotoxic risks in human primary hematopoietic cells.

**Funding:** DFG

For in vivo analysis a murine model of human hematopoietic engraftment and differentiation (humanized mouse model) utilizing the transplantation of human cord blood derived progenitor/stem cell populations into severely immunodeficient NOD/SCID/IL2r $\alpha$ <sup>null</sup> mice will be used. We will evaluate the suitability of this model for the assessment of clonal fate and insertional site effects following retroviral gene transfer into human HSCs by comparing a panel of vectors highly varying in their transformation potential in murine models.

In addition, we aim to establish an in vitro assay based on the replating and transformation capacity of human hematopoietic progenitor/stem cells similar to the murine In Vitro Immortalisation (IVIM) Assay developed by U. Modlich. Again Lin-/CD34<sup>+</sup> cord blood derived cells will be utilized.

Following transduction the cells will be subjected to three different culture protocols to establish the best way to achieve immortalization/transformation: i) in analogy to the murine IVIM cells will be expanded for two weeks and thereafter plated onto 96 well plates in limiting dilution; ii) the clonal diversity of mass cultures set up with different cytokines will be assessed by repeated DNA sampling for LM PCRs; iii) serial cultures in semisolid medium will be set up.

In the second phase of the project, we will utilize these two models to investigate clonal fate and insertional mutagenesis following retroviral gene transfer in the context of growth factor mediated *in vitro* expansion of hematopoietic cells by SCF, TPO, IGFBP2, FGF-I, and Angptl5. This technology could have profound impacts on the future development of hematopoietic gene therapy, however, clearly increases the proliferative stress exerted on transduced stem cell clones and therefore may facilitate stem cell exhaustion or cause other side effects such as lineage skewing or genetic instability. This project will furthermore give insights into differences in transformation between human and murine cells.

### **Time schedule**

1. Bone marrow transplantation experiments of gene modified cord blood in NOD/SCID/IL2r $\alpha$ <sup>null</sup> mice
2. Analysis of clonal distribution and development in transplanted mice by ligation mediated (LM) PCR

### **Group Members:**

**Department of Experimental Hematology:** C. Baum (head of department), U. Modlich (group leader), D. Heckl, M. Rothe, S. Wolf (PhD students), S. Knöß, J. Krause (technicians) and 27 further colleagues in the Department of Experimental Hematology

**AG Reprogramming:** T. Moritz (head of group), J. Jagielska (postdoc), H. Sryvidiameena, N. Lachmann, N. Pfaff (Ph. D. students), D. Lüttge (technician)

### **Key References for project**

1. Hacein-Bey-Abina S, Garrigue A, Wang GP, et al. Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1. *J Clin Invest* 2008;118(9):3132-42.
2. Cattoglio C, Facchini G, Sartori D, et al. Hot spots of retroviral integration in human CD34+ hematopoietic cells. *Blood* 2007;110(6):1770-8.
3. Piacibello W, Bruno S, Sanavio F, et al. Lentiviral gene transfer and *ex vivo* expansion of human primitive stem cells capable of primary, secondary, and tertiary multilineage repopulation in NOD/SCID mice. *Nonobese diabetic/severe combined immunodeficient*. *Blood* 2002;100(13):4391-400.
4. Zhang CC, Kaba M, Iizuka S, Huynh H, Lodish HF. Angiopoietin-like 5 and IGFBP2 stimulate *ex vivo* expansion of human cord blood hematopoietic stem cells as assayed by NOD/SCID transplantation. *Blood* 2008;111(7):3415-23.
5. Hahn WC, Weinberg RA. Rules for making human tumor cells. *N Engl J Med*. 2002 Nov 14;347(20):1593-603.

### **Own references:**

1. Bardenheuer W, Lehmborg K, Rattmann I, Brueckner A, Schneider A, Sorg UR, Seeber S, **Moritz T**, Flasshove M (2005) Resistance to cytarabine and gemcitabine and *in vitro* selection of transduced cells after retroviral expression of cytidine deaminase in human hematopoietic progenitor cells. *Leukemia* 19: 2281-2288
2. Rattmann I, Kleff V, Sorg UR, Bardenheuer W, Brueckner A, Hilger RA, Opalka B, Seeber S, Flasshove M, **Moritz T** (2006) Gene transfer of cytidine deaminase protects myelopoiesis from cytidine analogs in an *in vivo* murine transplant model. *Blood* 108: 2965-2971

3. Sorg U, Kleff V, Fanaei S, Schumann A, Moellmann M, Opalka B, Thomale J, **Moritz T** (2007) O<sup>6</sup>-methylguanine-DNA-methyltransferase (MGMT) gene therapy targeting haematopoietic stem cells: Studies addressing safety issues. *DNA Repair* 6: 1197-1209
4. Dürig J, Ebeling P, Grabellus F, Sorg UR, Möllmann M, Schütt P, Göthert J, Sellmann L, Seeber S, Flasshove M, Dührsen U, **Moritz T** (2007) A novel nonobese diabetic/severe combined immunodeficient xenograft model for chronic lymphocytic leukemia reflects important clinical characteristics of the disease. *Cancer Research* 67: 8653-8661
5. Kleff V, Sorg UR, Bury C, Bury C, Suzuki T, Rattmann I, Jerabek-Willemsen M, Poremba C, Flasshove M, Opalka B, Trapnell B, Dirksen U, **Moritz T** (2008) Gene therapy of  $\alpha$ c-deficient pulmonary alveolar proteinosis ( $\alpha$ c-PAP): Studies in a murine *in vivo* model. *Mol Ther* 16: 757-64
6. Milsom MD, Jerabek-Willemsen M, Harris CE, Schambach A, Broun E, Bailey J, Jansen M, Schleimer D, Nattamai K, Wilhelm J, Watson A, Geiger H, Margison GP, **Moritz T**, Baum C, Thomale J, Williams DA (2008) Reciprocal relationship between very high O<sup>6</sup>-methylguanine-DNA methyltransferase P140K expression level and chemoprotection/selection of hematopoietic stem cells. *Cancer Res* 68: 6171-80
7. **Modlich U**, Schambach A, Brugman MH, Wicke DC, Knoess S, Li Z, Maetzig T, Rudolph C, Schlegelberger B, Baum C (2008) Leukemia induction after a single retroviral vector insertion in Evi1 or Prdm16. *Leukemia* 22(8):1519-28
8. **Modlich U**, Bohne J, Schmidt M, von Kalle C, Knoess S, Schambach A and Baum C (2006) Cell culture assays reveal reduced insertional genotoxicity of self-inactivating retroviral vectors. *Blood* 15;108(8):2545-53
9. Kustikova O, Fehse B, **Modlich U**, Yang M, Dullmann J, Kamino K, von Neuhoff N, Schlegelberger B, Li Z, Baum C (2005) Clonal dominance of hematopoietic stem cells triggered by retroviral gene marking. *Science* 308(5725):1171-4.
10. **Modlich U**, Kustikova OS, Schmidt M, Rudolph C, Meyer J, Li Z, Kamino K, von Neuhoff N, Schlegelberger B, Kuehlcke K, Bunting KD, Schmidt S, Deichmann A, von Kalle C, Fehse B, Baum C (2005) Leukemias following retroviral transfer of multidrug resistance 1 (MDR1) are driven by combinatorial insertional mutagenesis. *Blood* 105(11):4235-46

## 11.)

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### Engagement in the MD/PhD program or HBRS:

Supervisor: X Name of student(s): Matthewos Tessema, Matthias Christgen  
Lecturer: X; PhD Kommission: ; No, not yet:

**Animal experiments involved:**  yes  no

### Research focus:

Our research focuses on the analysis of epigenetic aberrations in human malignancies with a focus on breast and liver cancer. Qualitatively and also quantitatively epigenetic aberrations are as important as genetic alterations for the development and progression of human malignancies.

Methodologically we focus on the analysis of primary human tissue specimens in order to identify molecular alterations which are not confounded by experimental conditions. Since the methods available for the analysis of DNA methylation and chromatin modifications are still evolving our efforts are also devoted to methodological improvements in the field of epigenetics.

### Specific project and methods applied:

**Title:** *Epigenetic regulation of microRNA gene expression in human hepatocellular carcinoma*

**Aims:** Aberrations of DNA methylation patterns regularly occur in human malignancies complementing other genetic aberrations by epigenetic silencing of genetic information. In previous studies we could show, that human hepatocellular carcinoma exhibits quantitatively and qualitatively altered DNA methylation patterns. Interestingly, potential pre-cancerous conditions like hemochromatosis already revealed identical aberrations of gene methylation. Furthermore, we found that microRNA genes, a new class of epigenetic regulators, are themselves targets of abnormal gene silencing by DNA methylation.

Using state-of-the-art quantitative high-resolution DNA methylation analysis technology we will figure out which sets of genes are affected in different types, grades and etiologic backgrounds of hepatocellular cancer. Referring to our findings in hemochromatosis we will investigate whether the causative agent of liver cell damage leading to cirrhosis and subsequently cancer causes a specific methylation signature.

The methylation analysis will focus on the identification of differentially methylated regions regulating the expression of microRNA genes. Detailed sequence analyses and functional assays will be employed for further characterization of these differentially methylated regions.

### Funding: DFG

The development and progression of malignant tumours is not only caused by genetic defects but also by epigenetic aberrations of chromatin structure (i.e., DNA methylation and histone modification). Alterations in DNA methylation patterns are a frequent and very early event in tumour evolution with a wide spectrum of diagnostic, prognostic and predictive applications. (1, 2) In fact, evidence suggests that the progression of aggressive cancers can be driven under certain circumstances by epigenetic events without genomic instability. (3) Morphologically and clinically different tumour subtypes show characteristic differences in DNA methylation patterns and "epigenetic" drugs targeting these aberrations are already in clinical trials. (4)

Current approaches for DNA methylation profiling of human biopsies have to be improved in terms of resolution, cost-effectiveness and through-put. The application of ultra deep-sequencing technologies for methylation analysis will lead to a much more complete and accurate picture of aberrations in DNA methylation in human malignancies. It offers the combination of single CpG-resolution with exact quantification in a high-throughput format.

A few pioneering studies have shown the feasibility and the potential of the "deep sequencing" approach in the field of DNA methylation analysis. These studies focused on lymphoma and breast cancer analyzing classical tumour suppressor genes (5) or specific chromosomal regions. (6) The class of microRNA genes or liver samples have not been analyzed so far using this innovative approach. For the analysis of pre-selected target regions amplicon sequencing using 454™ technology offers the best solution currently available because of the longest reading length (> 300 bp). (7)

In addition, this approach offers the possibility to detect a potentially present genetic heterogeneity in the differentially methylated regions. This might lead to new insights into the susceptibility of genomic regions to *de novo* methylation.

1. Coleman WB, Rivenbark AG (2006) Quantitative DNA methylation analysis: the promise of high-throughput epigenomic diagnostic testing in human neoplastic disease. *J Mol Diagn*; 8:152-156.
2. Iacobuzio-Donahue CA (2008) Epigenetic Changes in Cancer. *Annu Rev Pathol*; Oct 7 [Epub ahead of print].
3. McKenna ES, Roberts CW (2009) Epigenetics and cancer without genomic instability. *Cell Cycle*; 8:23-26.
4. Issa JP (2007) DNA methylation as a therapeutic target in cancer. *Clin Cancer Res*; 13: 1634-1637.
5. Taylor KH, Kramer RS, Davis JW, Guo J, Duff DJ, Xu D, Caldwell CW, Shi H (2007) Ultradeep bisulfite sequencing analysis of DNA methylation patterns in multiple gene promoters by 454 sequencing. *Cancer Res*; 67:8511-8518.
6. Korshunova Y, Maloney RK, Lakey N, Citek RW, Bacher B, Budiman A, Ordway JM, McCombie WR, Leon J, Jeddloh JA., McPherson JD (2008) Massively parallel bisulphite pyrosequencing reveals the molecular complexity of breast cancer-associated cytosine-methylation patterns obtained from tissue and serum DNA. *Genome Res*; 18: 19-29.
7. Rothberg JM, Leamon JH (2008) The development and impact of 454 sequencing. *Nat Biotechnol*; 26:1117-1124.

The long term goal of this project is the elucidation of the interplay of epigenetics, genetics, and etiology in the development of human hepatocellular carcinoma and the identification of molecular defects causing aberrant hypermethylation.

In order to achieve this goal the following questions will be addressed:

1. Can human HCC be subclassified by differential epigenetic changes?
2. Which epigenetic aberrations are present already in pre-cancerous conditions and do these aberrations reflect the aetiology of this condition and indicate the risk of transformation?
3. What are the functional consequences of differential microRNA gene methylation in primary human HCC?
4. Are the differentially methylated regions characterized by specific sequence variants or motifs indicating the likelihood and extent of aberrant methylation?
5. Do defects in the RNA processing machinery complement epigenetic mechanisms in primary human HCC?

We envisage that this work will contribute to a better understanding of the causes and consequences of epigenetic aberrations in human hepatocellular carcinoma and the role and regulation of non-protein-coding RNA genes in human tumour development including the perspective of new diagnostic, prognostic, or predictive markers and potential therapeutic targets.

### **Group Members:**

Anna Wüstefeld, Post Doc; Cord Albat, PhD student; Clemens Bockmeyer, MD; Matthias Christgen, MD, PhD; Jenny Weidner, Diploma student; David Albrecht, BSc student; Britta Hasemeier, research assistant; Jeanette Poczka, technician

## Key References for the project

See above ref. 1 – 7 and:

**Lehmann U\***, Hasemeier B, Christgen M, Müller M, Römermann D, Länger F, Kreipe H (2008). Epigenetic inactivation of microRNA gene hsa-mir-9-1 in human breast cancer. **J Pathol**, 214:17-24

Brakensiek K, Wingen LU, Länger F, Kreipe H, **Lehmann U\*** (2007). Quantitative high resolution CpG island mapping using Pyrosequencing reveals disease-specific methylation patterns of the *p15<sup>INK4b</sup>* gene in Myelodysplastic syndrome and Myeloid Leukemia. **Clin Chem** 53, 17-23

**Lehmann U\***, Wingen LU, Brakensiek K, Wedemeyer HH, Becker T, Heim A, Metzger K, Hasemeier B, Kreipe H, Flemming P (2007). Epigenetic defects of hepatocellular carcinoma are already found in non-neoplastic liver cells from patients with hereditary haemochromatosis. **Hum Mol Genet**, 16:1335-1342

**Lehmann U\***, Berg-Ribbe I, Wingen LU, Brakensiek K, Becker T, Klemptner J, Schlegelberger B, Kreipe H, Flemming P (2005). Distinct Methylation Patterns of Benign and Malignant Liver Tumors Revealed by Quantitative Methylation Profiling. **Clin Can Res** 11, 3654-3660

## Own references (2005 - 2009):

(\*: corresponding author)

Kocks JR, Adler H, Danzer H, Hoffmann K, Jonigk D, **Lehmann U**, Förster R\*. Chemokine receptor CCR7 contributes to a rapid clearance of MHV-68 from the lung while bronchus-associated lymphoid tissue harbours virus during latency **J Immunol**, *in press*

Zhao F, Vermeer B, **Lehmann U**, Kreipe H, Manns MP, Korangz F, Greten TF\*. Identification of a novel pancreatic tumor antigen, which elicits antibody responses in patients with pancreatic carcinoma. **Immunology**, *in press*

Singh AK, Riederer B, Krabbenhöft A, Rausch B, Bonhage J, **Lehmann U**, de Jonge HR, Donowitz M, Yun C, Weinman EJ, Kocher O, Hogema BM, Seidler U\* (2009). Differential roles of NHERF1, NHERF2, and PDZK1 in regulating CFTR-mediated intestinal anion secretion in mice. **J Clin Invest**, 119:540-550

Christgen M\*, Bruchhardt H, Hadamitzky C, Rudolph C, Steinemann D, Gadzicki D, Hasemeier B, Römermann D, Focken T, Krech T, Ballmaier M, Schlegelberger B, Kreipe H, **Lehmann U** (2009). Comprehensive genetic and functional characterization of IPH-926: A novel CDH1-null tumor cell line from human lobular breast cancer. **J Pathol**, 217:620-632

Gadzicki D\*, Schubert A, Milde S, **Lehmann U**, Fischer C, Steinemann D, Lück HJ, Kreipe H, Schlegelberger B. The impact of breast cancer morphology on the prediction of a BRCA1 mutation. **Can Genet Cytogenet**, 189:105-11

Hasemeier B, Christgen M, Kreipe H, **Lehmann U\*** (2008). Reliable microRNA profiling in routinely processed formalin-fixed paraffin-embedded breast cancer specimens using fluorescence labelled bead technology. **BMC Biotechnology**, 8:90

Christgen M\*, Bruchhardt H, Ballmaier M, Krech T, Länger F, Kreipe H, **Lehmann U** (2008). KAI1/CD82 is a novel target of estrogen receptor-mediated gene-repression and down-regulated in primary human breast cancer. **Int J Cancer**, 123: 2239-2246

Nickel N, Kempf, T, Tapken H, Laenger F, **Lehmann U**, Golpon H, Wilkins M, Gibbs SJ, Hoepfer MM\*, Wollert KC\* (2008). Growth differentiation Factor-15 as a Prognostic Indicator in Patients with Idiopathic Pulmonary Arterial Hypertension **Am J Resp Crit Care Med**, 178: 534-541

Römermann D, Hasemeier B, Metzger K, Göhring G, Schlegelberger B, Länger F, Kreipe H, **Lehmann U\*** (2008). Global increase in DNA methylation in patients with myelodysplastic syndrome (MDS) **Leukemia**, 22: 1954-1956

Ciesek S, Helfritz FA, **Lehmann U**, Becker T, Strassburg CP, Neipp M, Ciner A, Fytili P, Tillmann HL, Manns MP, Wedemeyer H\* (2008). Persistence of occult hepatitis B after explantation of the primarily infected liver. **J Inf Dis**, 197: 355-360

**Lehmann U\***, Hasemeier B, Christgen M, Müller M, Römermann D, Länger F, Kreipe H (2008). Epigenetic inactivation of microRNA gene hsa-mir-9-1 in human breast cancer. **J Pathol**, 214:17-24

Jonigk D, **Lehmann U**, Stucht S, Wilhelm M, Haverich A, Kreipe H, Mengel M\* (2007). VEGF-alpha promotes recipient derived neoangiogenesis in Quilty-lesions of cardiac allografts. **Transplantation**, 84:1335-1342

Christgen M\*, Ballmaier M, Bruchhardt H, von Wasielewski R, Kreipe H, **Lehmann U** (2007). Identification of a distinct side population of cancer cells in the Cal-51 human breast carcinoma cell line. **Mol Cell Biochem**, 306:201-212

- Lehmann U\***, Wingen LU, Brakensiek K, Wedemeyer HH, Becker T, Heim A, Metzsig K, Hasemeier B, Kreipe H, Flemming P (2007). Epigenetic defects of hepatocellular carcinoma are already found in non-neoplastic liver cells from patients with hereditary haemochromatosis. **Hum Mol Genet**, 16:1335-1342
- Rippberger T, von Neuhoff N, Kamphues K, Emura M, **Lehmann U**, Tauscher M, Schraders M, Groenen P, Skawran B, Rudolph C, Callet-Bauchu, van Krieken JHJM, Schlegelberger B, Steinemann D\* (2007). Promoter methylation of PARG1, a novel candidate tumor suppressor gene in mantle cell lymphoma. **Haematologica**, 92:460-468
- Lechel A, Holstege H, Begus Y, Schienke A, Kamino K, **Lehmann U**, Kubicka S, Schirmacher P, Jonkers J, Rudolph KL (2007). Telomerase deletion limits progression of p53-mutant hepatocellular carcinoma with short telomeres in chronic liver disease. **Gastroenterology**, 132:1465-1475
- Steimle C, **Lehmann U**, Temerinac S, Goertler PS, Kreipe H, Meinhardt G, Heimpel H, Pahl HL\* (2007). Biomarker analysis in polycythemia vera under interferon-alpha treatment: clonality, EEC, PRV-1, and JAK2 V617F. **Ann Hematol**, 86, 239-44
- Brakensiek K, Wingen LU, Länger F, Kreipe H, **Lehmann U\*** (2007). Quantitative high resolution CpG island mapping using Pyrosequencing reveals disease-specific methylation patterns of the *p15<sup>INK4b</sup>* gene in Myelodysplastic syndrome and Myeloid Leukemia. **Clin Chem** 53, 17-23
- Skokowa J\*, Cario G, Uenal M, Schambach A, Germeshausen M, Battmer K, Zeidler C, **Lehmann U**, Eder M, Baum C, Grosschedl R, Stanulla M, Scherr M, Welte K\* (2006). LEF-1 is crucial for neutrophil granulocytopenia and is abrogated in congenital neutropenia. **Nature Med** 12, 1191-1197
- Timmerbeul I, Garrett-Engele GM, Kossatz U, Chen X, Firpo E, Grünwald V, Kamino K, Wilkens L, **Lehmann U**, Buer J, Geffers R, Kubicka S, Manns MP, Porter PL, Roberts JM, Malek NP\* (2006). Testing the importance of p27 degradation by the SCF Skp2 pathway in murine models of lung and colon cancer. **Proc Natl Aca Sci USA** 38, 14009-14014
- Schade U, Bock O, Jaeger A, Buesche G, **Lehmann U**, Kreipe H\* (2006). Bone marrow-infiltration pattern in chronic lymphocytic leukemia (B-CLL) is related to IgVH mutation status and expression of the 70-kD zeta-associated protein. **Hum Pathol** 37, 1153-1161
- Bröcker V, Länger F, Fellous TG, Mengel M, Brittan M, Brecht M, Milde S, Welte T, Eder M, Haverich A, Alison MR, Kreipe H, **Lehmann U\*** (2006). Fibroblasts of Recipient Origin Contribute to Bronchiolitis Obliterans in Human Lung Transplantation. **Am J Resp Crit Care Med** 173, 1276-1282
- Stange DE, Radlwimmer B, Schubert F, Traub F, Pich A, Lejeune A, Toedt G, Mendrzyk F, **Lehmann U**, Eils R, Kreipe H, Lichter P\* (2006). Chromosomal aberrations on 1q and 16p discriminate histological and genetic subgroups of invasive breast cancer. **Clin Can Res** 12, 345-352
- Akdere F, Bock O, **Lehmann U**, Serinsöz E, Haverich A, Kreipe H, Mengel M\* (2005). Quantitative mRNA Expression Analysis of Co-Stimulatory Molecules in Sequential Biopsies from Heart Allografts. **Transpl Int** 18, 1197-1202
- Brakensiek K, Länger F, Kreipe H, **Lehmann U\*** (2005). Absence of *p21<sup>CIP1</sup>*, *p27<sup>KIP1</sup>* and *p57<sup>KIP2</sup>* methylation in MDS and AML. **Leuk Res**, 29, 1357-1360
- Brakensiek K, Länger F, Schlegelberger B, Kreipe H, **Lehmann U\*** (2005). Hypermethylation of the suppressor of cytokine signalling-1 (*SOCS-1*) in myelodysplastic syndrome. **Br J Haematol**, 130, 209-217
- Lehmann U\***, Berg-Ribbe I, Wingen LU, Brakensiek K, Becker T, Klemptner J, Schlegelberger B, Kreipe H, Flemming P (2005). Distinct Methylation Patterns of Benign and Malignant Liver Tumors Revealed by Quantitative Methylation Profiling. **Clin Can Res** 11, 3654-3660
- Serinsöz E, Bock O, Kirsch T, Haller H, **Lehmann U**, Kreipe H, Mengel M\* (2005) Compartment-specific Quantitative Gene Expression Analysis after Laser Microdissection from Archival Renal Allograft Biopsies. **Clinical Nephrology**, 63, 193-201
- Länger F, Dingemann J, Kreipe H, **Lehmann U\*** (2005) Up-regulation of DNA methyltransferases DNMT1, 3A, and 3B in myelodysplastic syndrome. **Leuk Res**, 29, 325-329
- Tessema M, Länger F, Bock O, Seltsam A, Metzsig K, Hasemeier B, Kreipe H, **Lehmann U\*** (2005). Down-regulation of the *IGF-2/H19* locus during normal and malignant hematopoiesis is independent of the imprinting pattern. **Int J Oncol** 26, 499-507

## 12.)

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### Engagement in the MD/PhD program or HBRS:

Supervisor: x Name of student(s): Katja Kochrube  
Lecturer: x; PhD Kommission: ; No, not yet: x

**Animal experiments involved:** x yes  no

### Research focus:

The immune system can be divided into two parts – the innate and the adaptive immune system. The innate immune system recognizes pathogens with a limited number of conserved germ-line encoded receptors and is able to react rapidly to initially control an infection. Cells of the adaptive immune system (antibody producing B cells and immunomodulatory T cells) on the other hand are characterized by a huge diversity of unique receptors which are assembled differently in every cell during ontogeny. Thus, only a few numbers of specific cells exist originally in the body and the reaction of the adaptive immune system requires a certain time period before becoming effective. However, a class of B cells – B1 cells - exist that exhibit properties of the innate immune system. Although their antibody receptor requires assembly during ontogeny, much like the receptors of the other classes of B cells, their receptor repertoire is rather limited and is mostly directed against conserved antigenic structures on common pathogens. In addition, they are able to react rapidly to antigen challenge by antibody secretion and do not require the help of modulatory T cells as the other B cell population (B2 cells) does.

B1 cells can be divided into B1a and B1b cells. Such cells are the dominating B cell population of body cavities although a small population of B1a cells is also found in spleen. They are responsible for the production of so-called natural antibodies i.e. antibodies that are found in the body without antigen challenge. Such antibodies have essential tasks at the initiation of immune responses but strangely enough some of them show low reactivity against self-antigen and they could be even multi-specific i.e. specific for several antigens.

In normal mice B1 cells are found only in rather low numbers. Therefore, we have refrained to a model system of a transgenic mouse - called the L2 mouse. These mice are transgenic for a  $\lambda$  immunoglobulin light (L) chain that can be easily distinguished from the normal  $\kappa$  L chain of B cells. For still unknown reasons, the extremely high expression of the  $\lambda$  transgene in B cells results in a mouse of which the B cell compartment is dominated by B1 cells. This mouse line has facilitated studies on the physiology and the antibody-receptor repertoire of B1 cells. Studies done in our group showed an extremely limited B cell receptor repertoire of B1 cells in the peritoneal cavity of such mice that was dependent on the presence of the spleen. Thus the spleen might provide survival factors or simply the architecture for limited self-reaction and B1 cell propagation. Similarly, the gut flora might be essential for the maintenance of the antibody receptor repertoire of such cells.

### Specific project and methods applied:

**Title:** *Migration of antigen-specific B1 cells*

**Aims:** When applying the L2 mouse model to study the physiology we noticed that splenectomy resulted in the loss of certain antigen specificities in the peritoneal cavity amongst the B1 cells while

others were undisturbed. The reason for this difference might lie in the differential migratory pattern of B1-cells that are specific for different antigens. Thus, the present project intends to characterize the migratory behavior of B1 cells and whether B1 cells with different antigen specific receptors migrate differently.

- Therefore, first the antigen specificities of L2 mouse derived B1 cells from spleen and the peritoneal cavity will be established. For this libraries will be established from the cDNA of sorted B1 cells. Then, using established expression systems and screening protocols, various antigens will be tested.
- Oral application of lipopolysaccharide (LPS) a cell wall component of Gram-negative bacteria results in the migration of many B1 cells from the peritoneal cavity to the spleen. Therefore, the remaining and the migrating B1 cells will be tested with reagents, that detect certain B1 cell associated specificities like PtC, Dextran etc including specificities that were discovered with the above screening protocol. In addition, novel reagents will be established that recognize the binding sites of particular antibodies (so-called anti-idiotypes, one of which has been already established and characterized). Such reagents will facilitate the analysis of specificities and the migration pattern of B1 cells expressing them. In addition, the surface marker phenotype of the migratory and the residual B1 cells will be compared by flow cytometry, since this will give indications of the activation status of such B cells.
- The above experiments will be extended to infection experiments. To this end, invasive and non-invasive *Salmonella typhimurium* strains as Gram-negative and *Listeria monocytogenes* as Gram-positive bacterial pathogen will be employed. We expect that infection will induce migration of B1 cells. Therefore, B1 cell associated specificities in spleen and peritoneal cavity will be tested including specificities against the applied pathogen. A still enigmatic aspect of B1 cell physiology is that often migration of B1 cells is found that are not specific for the particular pathogen. Therefore, B1 cells might have additional roles during an early immune response besides production of specific antibodies. Hence, the cytokine expression pattern of such cells will be established besides their activation state and antigen specificity.
- B1 cells usually do not proliferate. However, after migration to the spleen B1 cells were shown to expand and differentiate to antibody secreting plasma cells. In principle, this should result in a loss of B1 cells carrying such specificities. An unlikely scenario! Therefore, the spleen and the peritoneal cavity should be tested for the recovery of such specific B1 cells by employing the new reagents and the known antigen specificities. In addition, such B1 cells will be tested for proliferation *in vivo* since B1 cells usually do not proliferate under normal circumstances.
- The contribution of B1 cells to the gut-associated-immune system is still controversially discussed. From our own studies it is clear that B1 cells can contribute to the plasma cell pool in the lamina propria of the intestine and to IgA antibodies in the gut lumen. However, their competitiveness to B2 cells was not studied. Thus, it is still unclear whether under normal conditions B1 cells can compete with B2 cells for niches in the intestine. Therefore, in the present project the migratory capacity of various B1 cells to the gut should be examined. To this end different populations of B1 cells will be conditioned *in vitro* to migrate to the intestine and transferred into lymphopenic or normal mice. Subsequently, their competitive strength and the specificities of the gut-homing B1 cells will be established.

### **Techniques:**

The project is based on quite a number of demanding state of the art techniques. It will start with high speed cell sorting and cloning of the antigen receptor encoding cDNAs. High through put screening and sequencing and subsequent cytology and histology for characterization of specificities will be followed by purification of antibodies and immunization for anti-idiotypic antibodies. Specificities will be detected *ex vivo* by ELISPOT and ELISA and cells will be characterized by flow cytometry using 6 or more colors simultaneously. *In vivo*, B1 cell proliferation will be assessed by flow cytometry or histology and the cytokine pattern will be established by micro expression arrays and confirmed by qRT-PCR and intracellular staining.

**Funding:** *BMBF*

## **Time schedule**

1. Repertoire studies, LPS activation
2. Infection, Back migration or proliferation
3. Gut homing

## **Group Members:**

*Sandra Düber, Postdoc; Bishnudeo Roy, Postdoc*  
*Swati Shukla, PhD student*  
*Martina Krey, technician*

## **Key References for project**

- Allman, D., and S. Pillai. 2008. Peripheral B cell subsets. *Curr. Opin. Immunol.* 20:149-157.
- Baumgarth, N., J.W. Tung, and L.A. Herzenberg. 2005. Inherent specificities in natural antibodies: a key to immune defense against pathogen invasion. *Springer Semin. Immunopathol.* 26:347-362.
- Wardemann H., T. Boehm, N. Dear, and R. Carsetti. 2002. B-1a B cells that link the innate and adaptive immune responses are lacking in the absence of the spleen. *J. Exp. Med.* 195:771-780.
- Yang, Y., J.W. Tung, E.E.B. Ghosn, L.A. Herzenberg, and L.A. Herzenberg. 2007. Division and differentiation of natural antibody-producing cells in mouse spleen. *Proc. Natl. Acad. Sci. U. S. A.* 104:4542-4546.

## **Own references (mainly 2004-2006):**

- Kretschmer, K., H. Engel, and S. Weiss. 2002. Strong antigenic selection shaping the immunoglobulin heavy chain repertoire of B-1a lymphocytes in lambda 2(315) transgenic mice. *Eur. J. Immunol.* 32:2317-2327.
- Kretschmer, K., A. Jungebloud, J. Stopkowitz, B. Stoermann, R. Hoffmann, and S. Weiss. 2003. Antibody repertoire and gene expression profile: implications for different developmental and functional traits of splenic and peritoneal B-1 lymphocytes. *J. Immunol.* 171:1192-1201.
- Kretschmer, K., J. Stopkowitz, S. Scheffer, T.F. Greten, and S. Weiss. 2004. Maintenance of peritoneal B-1a lymphocytes in the absence of the spleen. *J. Immunol.* 173:197-204.
- Roy, B., S. Shukla, M. Lyszkiewicz, M. Krey, N. Viegas, S. Düber, and S. Weiss. 2009. Somatic hypermutation in peritoneal B1b cells. *Mol. Immunol.* 46:1613-1619.
- Roy, B., S. Shukla, B. Stoermann, E. Kremmer, S. Düber, and S. Weiss. 2009. Loss of lambda2(315) transgene copy numbers influences the development of B1 cells. *Mol. Immunol.* 46:1542-1550.

13.)

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Supervisor: x  Name of student(s): Ayesha Sultan

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**Animal experiments involved:** x  yes  no

**Research focus:**

Our group studies the functional role and regulation of newly identified ion transport proteins in the gastrointestinal tract. The goal is to better understand the complex regulation of the ion transport pathways that are involved in the secretory and absorptive functions of the gastric, intestinal and biliary epithelium, to define the molecular and cellular defects in genetic diseases in which these proteins are dysfunctional, and to find new targets for drug development for some of the most prevalent gastrointestinal diseases – acute and chronic diarrhea, inflammatory bowel disease, cystic fibrosis, peptic ulcer disease, and chronic pancreatitis

**Current Projects for a MDPHD thesis :**

**Background:** Acute flares of Crohn's disease and ulcerative colitis are accompanied by, diarrhea, which is often the most distressing symptom for the patient. Interestingly, glucocorticoids have a proresorptive effect in the intestine unrelated to their anti-inflammatory effect (Sellin & DeSoignie, 1985; Isenberg et al., 1987; Turnamian & Binder, 1989; Turnamian & Binder, 1989; Bastl et al., 1989; Sandle, 1991). Even during the inflammatory flare, glucocorticoids often rapidly ameliorate the diarrhea long before any effect is seen on the histological appearance of the mucosa (Binder & Ptak, 1970; Sandle et al., 1986). One potential molecular mechanism is the upregulation of the expression levels for both the Na<sup>+</sup>/H<sup>+</sup> exchanger NHE3, the transport protein responsible for electroneutral sodium absorption (Hoogerwerf et al., 1996; Yun et al., 2002; Yun et al., 1993) and the expression of the epithelial sodium channel ENaC (Schulz-Baldes et al., 2001). We have recently studied this question in a CD45RB<sup>high</sup> transfer colitis mouse model and found a very strong upregulation of NHE3 transcription, protein abundance, and functional activity, resulting in a normalization of fluid absorption rates. We now want to study the effect of TNF- $\alpha$  antagonists on NHE3 expression and activity, fluid absorption and the mucosal inflammatory process and compare it to the effects of the steroids. We also want to study the alterations in the apical NHE3-PDZ-adaptor-multiprotein complexes during inflammation and healing. This will allow a better understanding of the molecular mechanisms of inflammatory diarrhea.

The *objectives of the study* are:

Now we want to find out how fast anti-TNF- $\alpha$  treatment can reverse the observed change in absorptive defect and in reduced NHE3 transport rate activation, and if there is a difference in reversibility when you give the antibody early vs. late in the inflammatory process. For this, we need to establish

1. mouse model with a well defined time course of inflammation.
2. look at the electrolyte and fluid transport *in vivo* before and after anti-TNF treatment.
3. study the changes in expression and function of electrolyte transport proteins in those mice and the changes that occur in the NHE3 multiprotein complexes during inflammation and healing by isolation from the brush border membrane of inflamed mouse intestine
4. reproduce these changes in cell culture.

3. Questions and goals:

1. Assessment of the changes in transport protein expression levels and their cellular localization during the development of Crohn's-like disease in a TNF- $\alpha$  overexpressing mouse model.
2. Assessment of the functional consequences for transport rates, regulation of transport, and barrier function, that are associated with a chronic increase in mucosal TNF- $\alpha$  levels.
3. Assessment of the effect of anti-TNF- $\alpha$  antibody treatment on the reversibility of the above-mentioned disturbances.
4. Parallel studies in biopsies from IBD patients before and after treatment with anti-TNF treatment will be performed.

### **Methods that will be used in the projects:**

*Organ and cell physiology in transgenic mice:*

Mini-Ussing-chamber techniques, pH-stat titration, isotope flux studies, confocal microscopy, Live-cell imaging with videoimaging and confocal laserscanning techniques in primary and transfected cells

*Cell culture experiments:*

Culture of gastrointestinal cells, single cell- and video-fluoromicroscopy, treatment of cultured cells with cytokines, measurement of changes in PDZ-adaptor complex formation by biochemical and immunological methods, measuring the change of transport function with fluorometric and electrophysiological techniques.

*Molecular biological and immunological techniques:*

recombinant DNA technology, site directed mutagenesis to construct CFTR mutants, adenovirus-and liposomal gene transfer, transfection of cell cultures, immunohistochemistry

### **Key references:**

Singh, A.K., Riederer, B., Krabbenhöft, A., Rausch, B., de Jonge, H., Donowitz, M., Weinman, E.J., Kocher, O., Hogema, B.M., and U. Seidler. Differential role for the PDZ proteins NHERF1, NHERF2 and PDZK1 in the regulation of CFTR-mediated intestinal anion secretion *in vivo*. J. Clin. Invest., epubl Feb 16th, 2009

Yeruva, S., Farkas, K., Hubricht, J., Rode, K., Riederer, B., Bachmann, O., Rakonczay, Z., Molnár, T., Nagy, F., Wedemeyer, J., Manns, M., Raddatz, D., Musch M., Chang E., Hegyi, P., and U. Seidler. Impaired intestinal Na<sup>+</sup>/H<sup>+</sup> exchanger isoform 3 transport function in ulcerative colitis may be related to loss of NHE3 adaptor protein NHE3, submitted to GASTROENTEROLOGY, PDF upon request.

- Broere, N., Chen, M., Cinar, A., Singh, A.K., Hillesheim, J., Riederer, B., Lünemann, M., Rottinghaus, I., Krabbenhöft, A., Engelhardt, R., Rausch, B. Weinman, E.J. Donowitz, M. Hubbard, A., Kocher, O., de Jonge, H.R., Hogema, B.M., Seidler, U. Defective jejunal and colonic salt absorption and altered NHE3 activity in PDZ-adaptor protein NHERF-1 deficient mice. *Pflügers Arch.* 457(5):1079-91, 2009
- Zheng, W., Kuhlicke, J.Jäckel, K., Eltschig, H.K., Singh, A.K., Sjöblem, M., Riederer, B., Weinhold, C., Seidler, U., Colgan, S.P., Karhausen, J. Hypoxia inducible facotr-1 (HIF1) mediated repression of cystic fibrosis transmembrane conductance regulator (CFTR) in the intestinal epithelium. *FASEB J.* 23(1):204-13, 2009
- Hillesheim, J. B. Riederer, B. Tuo, M. Chen, M. Manns, J. Biber, C. Yun, O. Kocher, und U. Seidler. Downregulation of small intestinal ion transport in PDZK1 (CAP70/NHERF3) deficient mice. *Pflügers Arch.* 2007 Jul;454(4):575-86, 2007
- Cinar, A., M. Chen, B. Riederer, O. Bachmann, M.P. Manns, O. Kocher und U. Seidler. NHE3 inhibition by cAMP and Ca<sup>2+</sup> but not hyperosmolarity is abolished in PDZ-protein PDZK1-deficeint murine enterocytes. *J. Physiol.* ;581(Pt 3):1235-46, 2007
- Seidler.U., H. Lenzen, A. Cinar, T. Tessema, und B. Riederer. Molecular mechanisms of disturbances electrolyte transport in intestinal inflammation. *Ann. N.Y.Acad. Sci* 1072:262-276, 2006
- Seidler, U., Singh, A.K. , Chen, M., Cinar, A., Bachmann, O., Zheng, W. Wang, J. Yeruva, S. reiderer, B. New insights into cystic fibrosis, secretory diarrhea and fructose-induced hypertension., *Exp Physiol.* 94(2):175-9, 2009
- Lamprecht, G., und U. Seidler. The emerging role of PDZ adapter proteins for regulation of intestinal ion transport. 2006. *Am J. Physiol.* 291(5):G766-77
- Barmeyer, C., Harren, M., Schmitz, H., Heinzl-Pleines, U., Mankertz, J., Seidler, U., Horak, I., Wiedenmann, B., Fromm, M., & Schulzke, J. D. (2004). Mechanisms of diarrhea in the interleukin-2-deficient mouse model of colonic inflammation. *Am.J.Physiol.* 286, G244-G252, 2004
- Bastl, C. P., Schulman, G., & Cragoe, E. J., Jr. (1989). Low-dose glucocorticoids stimulate electroneutral NaCl absorption in rat colon. *Am.J.Physiol* 257, F1027-F1038.
- Berin, M. C., McKay, D. M., & Perdue, M. H. (1999). Immune-epithelial interactions in host defense. *Am.J.Trop.Med.Hyg.* 60, 16-25.
- Binder, H. J. & Ptak, T. (1970). Jejunal absorption of water and electrolytes in inflammatory bowel disease. *J.Lab Clin.Med.* 76, 915-924.
- Field, M. (2003). Intestinal ion transport and the pathophysiology of diarrhea. *J.Clin.Invest.* 111, 931-943.

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### Engagement in the MD/PhD program or HBRS:

Supervisor: x  Name of student(s): Ayesha Sultan  
Lecturer: ; PhD Kommission: ; No, not yet:

**Animal experiments involved:** x  yes  no

### Research focus:

Our group studies the functional role and regulation of newly identified ion transport proteins in the gastrointestinal tract. The goal is to better understand the complex regulation of the ion transport pathways that are involved in the secretory and absorptive functions of the gastric, intestinal and biliary epithelium, to define the molecular and cellular defects in genetic diseases in which these proteins are dysfunctional, and to find new targets for drug development for some of the most prevalent gastrointestinal diseases – acute and chronic diarrhea, inflammatory bowel disease, cystic fibrosis, peptic ulcer disease, and chronic pancreatitis

### Current Projects for a MDPHD thesis :

#### **Background:**

CF epithelia display a reduction in  $\text{Cl}^-$ ,  $\text{HCO}_3^-$  and fluid secretion and an increase in salt and fluid absorption. The pathophysiological correlates are sticky, viscous mucoid secretions impacting ductal structures of multiple glandular organs and the intestine. This results in progressive glandular destruction, and increased susceptibility to bacterial infections of the affected epithelia. Recent data suggest that the defect in epithelial  $\text{HCO}_3^-$  secretion may have a particularly important role in the pathogenesis of these destructive changes.

The most frequent mutation in CF patients is the delta F508 “trafficking” mutant. Recent drug development strategies have focused on the potential „correction“ of this trafficking defect, and delta F508 molecules have successfully been integrated into the plasma membrane. However, it is unclear whether, and by what molecular mechanism, delta F508 can promote epithelial  $\text{HCO}_3^-$  secretion when expressed in the plasma membrane, and if this increased  $\text{HCO}_3^-$  secretion will affect the magnitude and quality of other secreted products such as mucus.

The *objectives of the study* are

1. to find out whether the delta F508 mutant promotes epithelial  $\text{HCO}_3^-$  secretion when expressed in the membrane,
2. to understand the molecular mechanisms of delta F508-augmented epithelial  $\text{HCO}_3^-$  secretion and

3. to establish mechanisms to enhance  $\text{HCO}_3^-$  secretion in delta F508 epithelia *in vitro* and *in vivo*

To clarify these question, the  $\text{HCO}_3^-$  secretory activity as well as mucus output will be measured in duodenal and colonic epithelium of transgenic mice *in vivo* and *in vitro*, as well in mucosa from CF patients, that express either no CFTR, the delta F508 mutant, or wt CFTR, in combination with one or several members of the SLC26 gene family of apical  $\text{Cl}^-/\text{HCO}_3^-$  exchangers. Some of the molecular details will be worked out in heterologous expression studies of the delta F508 mutant and the respective anion exchangers. These experiments will allow us to find out whether  $\text{HCO}_3^-$  exits via the delta F508 molecule itself, or whether delta F508 is able to augment  $\text{HCO}_3^-$  secretion via stimulation of apical  $\text{Cl}^-/\text{HCO}_3^-$  exchange. Depending on the results, we will test therapeutic strategies that aim specifically at increasing the  $\text{HCO}_3^-$  secretory activity of delta F508 expressing epithelia.

The project is broken to achieve several milestones:

**Milestone 1:** Establishing the role of delta-F508 in epithelial  $\text{HCO}_3^-$  secretion *in vivo*

**Milestone 2:** Determining the molecular mechanisms of delta-F508 augmented  $\text{HCO}_3^-$  transport

**Milestone 3:** Testing therapeutic strategies to increase  $\text{HCO}_3^-$  secretion in delta-F508 epithelia

#### **Methods that will be used in the projects:**

*Organ and cell physiology in transgenic mice:*

Mini-Ussing-chamber techniques, pH-stat titration, isotope flux studies, isolation and primary culture of gastrointestinal cells, measurement of secretion in single cells, single cell- and video-fluoromicroscopy, confocal microscopy,

Live-cell imaging with videoimaging and confocal laserscanning techniques in primary and transfected cells

*Molecular biological and immunological techniques:*

recombinant DNA technology, site directed mutagenesis to construct CFTR mutants, adenovirus-and liposomal gene transfer, transfection of cell cultures, immunohistochemistry

#### **Key references:**

- 1 Singh, A.K., Riederer, B., Krabbenhöft, A., Rausch, B., de Jonge, H., Donowitz, M., Weinman, E.J., Kocher, O., Hogema, B.M., und U. Seidler. Differential role for the PDZ proteins NHERF1, NHERF2 and PDZK1 in the regulation of CFTR-mediated intestinal anion secretion *in vivo*. J. Clin. Invest., epubl Feb 16th, 2009
- 2 Singh, A.K., Sjöblom, M., Zheng, W., Krabbenhöft, A., Riederer, B., Rausch, B., Manns, M.P., Soleimani, M., Seidler, U. CFTR and its key role in *in vivo* resting and luminal acid-induced duodenal  $\text{HCO}_3^-$  secretion. Acta Phys. (Lond.);193:357-65, 2008
- 3 Seidler, U., Singh, A.K., Chen, M., Cinar, A., Bachmann, O., Zheng, W. Wang, J. Yeruva, S. reiderer, B. New insights into cystic fibrosis, secretory diarrhea and fructose-induced hypertension., Exp Physiol. 2009 Feb;94(2):175-179
- 4 Quinton PM. Cystic fibrosis: impaired bicarbonate secretion and mucoviscidosis. Lancet 2008;372:415-417.
- 5 Guerra L, Fanelli T, Favia M, Riccardi SM, Busco G, Cardone RA, Carrabino S, Weinman EJ, Reshkin SJ, Conese M, Casavola V.  $\text{Na}^+/\text{H}^+$  exchanger regulatory factor isoform 1 overexpression modulates cystic fibrosis transmembrane conductance regulator (CFTR) expression and activity in human airway 16HBE14o- cells and rescues DeltaF508 CFTR functional expression in cystic fibrosis cells. J Biol Chem 2005;280:40925-40933.

- 6 Kwon SH, Pollard H, Guggino WB. Knockdown of NHERF1 enhances degradation of temperature rescued DeltaF508 CFTR from the cell surface of human airway cells. *Cell Physiol Biochem* 2007;20:763-772.
- 7 Wolde M, Fellows A, Cheng J, Kivenson A, Coutermarsh B, Talebian L, Karlson K, Piserchio A, Mierke DF, Stanton BA, Guggino WB, Madden DR. Targeting CAL as a negative regulator of DeltaF508-CFTR cell-surface expression: an RNA interference and structure-based mutagenetic approach. *J Biol Chem* 2007;282:8099-8109.
- 8 Yoo CL, Yu GJ, Yang B, Robins LI, Verkman AS, Kurth MJ. 4'-Methyl-4,5'-bithiazole-based correctors of defective delta F508-CFTR cellular processing. *Bioorg Med Chem Lett* 2008;18:2610-2614.
- 9 Seidler U, Blumenstein I, Kretz A, Viellard-Baron D, Rossmann H, Colledge WH, Evans M, Ratchliff R, Gregor M. A functional CFTR protein is required for mouse intestinal cAMP-, cGMP- and Ca(2+)-dependent HCO<sub>3</sub><sup>-</sup> secretion. *J Physiol* 1997;505:411-423.
- 10 Clarke LL, Harline MC. Dual role of CFTR in cAMP-stimulated HCO<sub>3</sub><sup>-</sup> secretion across murine duodenum. *Am J Physiol* 1998;274:G718-G726.
- 11 Poulsen JH, Fischer H, Illek B, Machen TE. Bicarbonate conductance and pH regulatory capability of cystic fibrosis transmembrane conductance regulator. *Proc Natl Acad Sci U S A* 1994;91:5340-5344.
- 12 Illek B, Fischer H, Machen TE. Genetic disorders of membrane transport. II. Regulation of CFTR by small molecules including HCO<sub>3</sub><sup>-</sup>. *Am J Physiol* 1998;275:G1221-G1226.
- 13 Reddy MM, Quinton PM. Selective activation of cystic fibrosis transmembrane conductance regulator Cl<sup>-</sup> and HCO<sub>3</sub><sup>-</sup> conductances. *JOP* 2001 Jul ;2(4 Suppl):212 -8.
- 14 Steward MC, Ishiguro H, Case RM. Mechanisms of bicarbonate secretion in the pancreatic duct. *Annu Rev Physiol* 2005;67:377-409.
- 15 Dorwart MR, Shcheynikov N, Yang D, Muallem S. The solute carrier 26 family of proteins in epithelial ion transport. *Physiology (Bethesda)* 2008;23:104-114.
- 16 Shcheynikov N, Wang Y, Park M, Ko SB, Dorwart M, Naruse S, Thomas PJ, Muallem S. Coupling modes and stoichiometry of Cl<sup>-</sup>/HCO<sub>3</sub><sup>-</sup> exchange by slc26a3 and slc26a6. *J Gen Physiol* 2006;127:511-524.
- 17 Shcheynikov N, Kim KH, Kim KM, Dorwart MR, Ko SB, Goto H, Naruse S, Thomas PJ, Muallem S. Dynamic control of cystic fibrosis transmembrane conductance regulator Cl<sup>-</sup>/HCO<sub>3</sub><sup>-</sup> selectivity by external Cl<sup>-</sup>
- 18 O'Reilly CM, Winpenny JP, Argent BE, Gray MA. Cystic fibrosis transmembrane conductance regulator currents in guinea pig pancreatic duct cells: inhibition by bicarbonate ions. *Gastroenterology*. 2000 Jun;118(6):1187-96.
- 19 Norez C, Noel S, Wilke M, Bijvelds M, Jorna H, Melin P, De Jonge H, Becq F. Rescue of functional delF508-CFTR channels in cystic fibrosis epithelial cells by the  $\alpha$ -glucosidase inhibitor miglustat. *FEBS Lett* 2006; 580: 2081-20

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**Engagement in the MD/PhD program or HBRs:**

Supervisor: X Name of student(s): Mudita Pincha

Lecturer: X; PhD Kommission: ; No, not yet:

**Animal experiments involved:** X yes  no

**Research focus:**

Research in my group is focused on the understanding and targeted manipulation of the immune system to be applied in the cure of human diseases. Our approach is to genetically program blood cells with designed viral vectors, such that they can be used in the treatment of diseases like cancer and chronic infections, where the immune responses have been severely abrogated. We utilize lentiviral vectors derived from HIV-1 that deliver ectopic expression of cytokines and antigens into hematopoietic precursors, which are then capable to self-differentiate into antigen presenting cells. This concept is currently being developed for immunotherapy of melanoma, leukemia and Hepatitis C.

**Specific project and methods applied:**

**Specific project and methods applied**

**Title:** *“Pre-clinical evaluation of self-differentiating and self-eliminating dendritic cells for melanoma immunotherapy“*

**Funding:** Rebirth; Deutsche Krebshilfe Stiftung (Pending grant proposal).

**Specific aims and work program (350 words):**

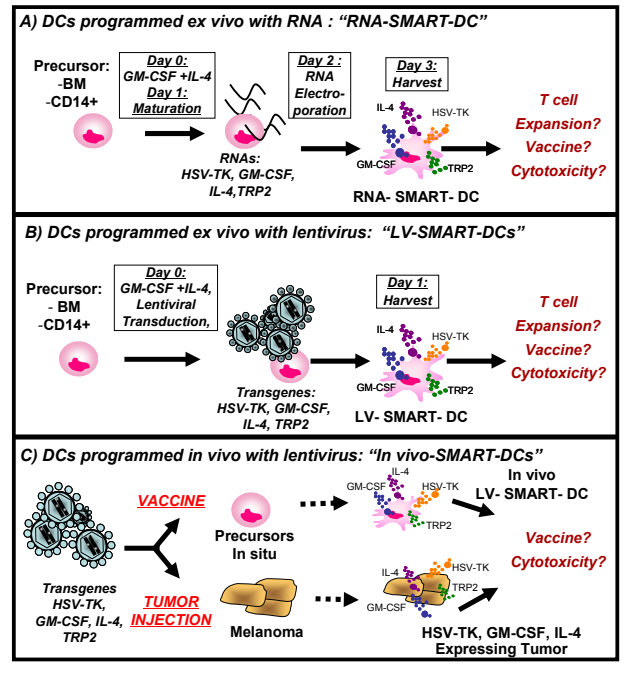
**Goals:** Dendritic cells (DCs) are potent immune adjuvants capable of enhancing weak antigenic responses against tumors and thus reverting tumor-induced anergy or tolerance. Therefore, their use in tumor immunology has been extensively explored. Nevertheless, large-scale *ex vivo* DC production in the laboratory remains highly costly and impractical in the clinic. A current goal of our laboratory is to learn how to simultaneously drive DCs to self-differentiate, present disease-specific antigens, and lead to tumor cytotoxic effects, such that more effective and less costly vaccines can be produced. Despite being historically the leading cancer population in the testing of dendritic cell vaccination, outcomes for metastatic melanoma have been so far at most modest and mortality remains high, warranting more practical and effective immuno/ cytotoxic therapy modalities

**Previous Work:** We have demonstrated in human and mouse systems that *ex vivo* transduction of DC precursors with lentiviral vectors (LVs) for production of Granulocyte Macrophage Colony Stimulating Factor (GM-CSF), Interleukin 4 (IL-4) and tumor antigens induced self-differentiation of potent therapeutic DC vaccines against melanoma (Koya, Kimura et al. 2007)\*. These cells were called “SMART”-DCs (Self-differentiated Myeloid-derived Antigen-presenting-cells Reactive against Tumors). We have also shown that LVs have a natural tropism to infect DC precursors *in vivo* leading to protective

antitumor immunization when used as vaccines (Kimura, Koya et al. 2007)\*. We have now developed and validated non-integrated tricistronic LV systems for co-expression of GM-CSF, IL-4 and HSV-TK. SMART-DCs programmed with these vectors were potent vaccines, susceptible to Ganciclovir.

**Time schedule:** We propose to advance towards pre-clinical testing of these novel modalities of SMART-DC vaccines. Initially, we will compare the feasibility and efficacy of two gene delivery approaches to generate SMART-DCs: RNA co-transfection, the current clinical state-of-the art modality (**Fig. 1A: 1<sup>st</sup> – 2<sup>nd</sup> year**) versus lentiviral transduction, experimental but a robust system (**Fig. 1B: 1<sup>st</sup>-2<sup>nd</sup> year**). We will develop non-integrating tetracistronic lentiviral vectors expressing the HSV-TK suicide gene, GM-CSF and IL-4 and the TRP2 melanoma antigen. TRP2 is a melanoma non-mutated antigen validated in the mouse and human systems (Paschen, Song et al. 2005). Developing new methods of *ex vivo* genetically programmed DC differentiation will yield important basic information, which we will also seek to apply towards a fully *in vivo* LV vaccine strategy (**Fig. 1C: 3<sup>rd</sup> year**). Thus, subsequently we will test non-integrating tetracistronic LVs as therapeutic vaccines. Our ultimate goal is to translate SMART-DCs and/or LVs combining immunotherapy and cytotoxicity effects clinically.

**Figure 1: Novel Vaccines to be evaluated: “RNA-SMART-DCs”, “LV-SMART-DCs” and “In vivo-SMART-DCs”.**



**GroupMembers:**

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## Key References for project

- Koya, R. C., T. Kimura, et al. (2007). "Lentiviral vector-mediated autonomous differentiation of mouse bone marrow cells into immunologically potent dendritic cell vaccines." Mol Ther **15**(5): 971-80.
- Kimura, T., R. C. Koya, et al. (2007). "Lentiviral Vectors with CMV or MHCII Promoters Administered In Vivo: Immune Reactivity Versus Persistence of Expression." Mol Ther **15**(7): 1390-9
- Lennerz, V., M. Fatho, et al. (2005). "The response of autologous T cells to a human melanoma is dominated by mutated neoantigens." Proc Natl Acad Sci U S A **102**(44): 16013-8
- Paschen, A., M. Song, et al. (2005). "Detection of spontaneous CD4+ T-cell responses in melanoma patients against a tyrosinase-related protein-2-derived epitope identified in HLA-DRB1\*0301 transgenic mice." Clin Cancer Res **11**(14): 5241-7.
- Schadendorf, D., S. Ugurel, et al. (2006). "Dacarbazine (DTIC) versus vaccination with autologous peptide-pulsed dendritic cells (DC) in first-line treatment of patients with metastatic melanoma: a randomized phase III trial of the DC study group of the DeCOG." Ann Oncol **17**(4): 563-70.

## Own references (mainly 2004-2006):

1. Prins, R.M., Craft N., Bruhn, K., Khan-Farooqi, H., Koya, R., Stripecke, R., Miller, J.F. and Liau, L.M. (2006). The Toll-like receptor agonist, imiquimod, enhances dendritic cell survival and promotes tumor antigen-specific T cell priming: relation to CNS anti-tumor immunity. **J. Immunology** 176(1):157-64.
2. Koya R., Kimura T., Ribas A., Rozengurt N., Lawson GW, Faure-Kumar E., Wang H-J, Herschmann H., Kasahara K. and Stripecke R (2007). Lentiviral vector-mediated autonomous differentiation of mouse bone marrow cells into immunologically potent dendritic cell vaccines. Mol Ther. 15(5):971-80
3. Kimura T., Koya RC., Anselmi L, Sternini C., Wang H., Comin-Anduix B., Prins R., Faure-Kumar E., Cui Y., Rozengurt N., Kasahara N. and Stripecke R (2007). Lentiviral Vectors with CMV or MHCII Promoters administered *in vivo*: Immune reactivity versus persistence of expression. Mol Ther. 15(7):1390-9.
4. Stripecke, R., Kasahara, N. Lentiviral and Retroviral Vector Systems. Cancer Drug Discovery and Development: Gene Therapy for Cancer. 2007. Dr. Kelly K. Hunt, ed.: 39-71
5. Köchling J, Prada J, Bahrami M, Stripecke R, Seeger K, Henze G, Wittig B, Schmidt M (2008). Anti-tumor effect of DNA-based vaccination and dSLIM immunomodulatory molecules in mice with Ph(+) acute lymphoblastic leukaemia. Vaccine 26;26(36):4669-4675.
6. Stripecke, R. Lentiviral vector-mediated genetic programming of mouse and human dendritic cells. Methods Mol. Biol. 2009; 506:139-58.

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**Engagement in the MD/PhD program or HBRs:**

Supervisor: X Name of student(s): Mudita Pincha

Lecturer: X; PhD Kommission: ; No, not yet:

**Animal experiments involved:** X yes  no

**Research focus:**

Research in my group is focused on the understanding and targeted manipulation of the immune system to be applied in the cure of human diseases. Our approach is to genetically program blood cells with designed viral vectors, such that they can be used in the treatment of diseases like cancer and chronic infections, where the immune responses have been severely abrogated. We utilize lentiviral vectors derived from HIV-1 that deliver ectopic expression of cytokines and antigens into hematopoietic precursors, which are then capable to self-differentiate into antigen presenting cells. This concept is currently being developed for immunotherapy of melanoma, leukemia and Hepatitis C.

**Specific project and methods applied:**

**Title:** *“Pre-clinical validation in a mouse model of dendritic cell vaccination against the FLT3-ITD leukemia signature“*

**Funding:** LOM or Deutsche José Carreras Leukämie-Stiftung e.V. (Pending)

**Specific aims and work program (350 words):**

Minimal residual disease (MRD) remains as the main obstacle to improve the long-term survival of leukemia patients. Induction of anti-leukemia immune responses during remission will be likely to promote rejection of residual leukemia cells and therefore cure. This project aims to stimulate autologous immune responses against MRD by genetically modifying antigen-presenting cells with an oncogene such that they will present a profile of putative antigenic proteins similar to the original leukemia to the immune system. Dendritic cells (DCs) are the most potent antigen-presenting cells in the body, and it has been recently shown that their frequency decreases significantly with aging. Therefore, in order to improve the survival of high-risk elderly leukemia patients who are not eligible for high intensity treatment modalities, a potential approach would be the adoptive transfer of autologous, ex vivo expanded DCs. Our group has recently developed a method of genetically programming monocytes with designed lentiviral vectors co-expressing tumor antigens, GM-CSF and IL-4, which can autonomously differentiate into long-lived and immunologically potent dendritic cells in vitro and in vivo (Koya, Weber et al. 2004) (Koya, Kimura et al. 2007). Since this DC genetic programming is readily achieved in one day and DCs self-differentiate subcutaneously, this method requires significant lower amounts of monocytes for DC production and bypasses the cell culture requirement, facilitating implementation in larger

cohorts of patients. We propose to advance towards pre-clinical development of these programmed DCs for prophylactic vaccination of elderly patients with Acute Myeloid Leukemia (AML). Our focus will be on patients carrying the FLT3-ITD mutation, which occurs in approximately 30% of AML cases and is one of the pivotal high-risk prognostic markers for AML at presentation and relapse. Our hypothesis is that FLT3-ITD co-expression in engineered DCs will lead to of a similar protein expression signature as present in the original FLT3-ITD leukemia cells. **In the context of a PhD project, the specific aims are:**

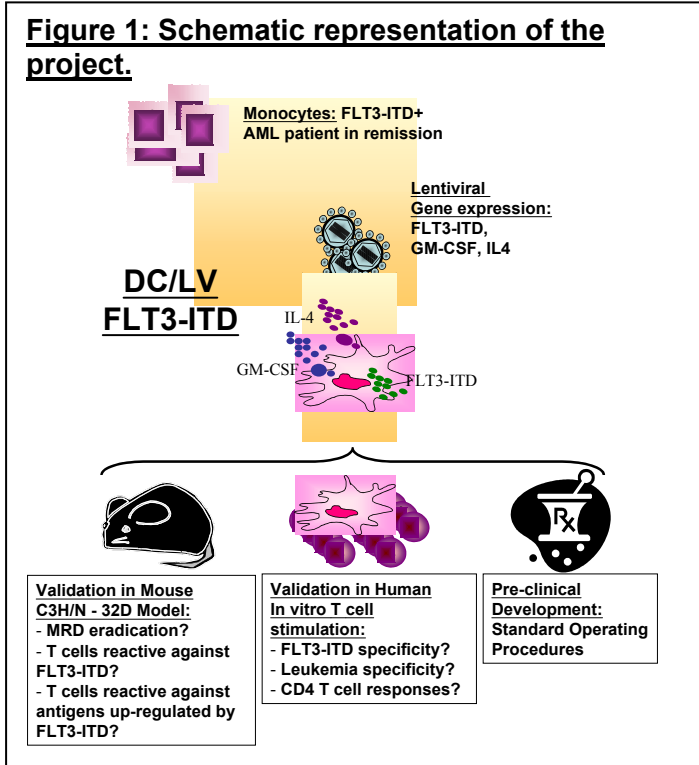
**Time Frame:**

**Aim 1:** To engineer safety-enhanced tetracistronic non-integrating lentiviral vectors for simultaneous co-expression of GM-CSF, IL-4 the suicide gene HSV-TK and prototypic FLT3-ITD oncogenes. Mouse bone marrow (BM) will be transduced with the lentiviral vectors to self-differentiate into DC/LV-FLT3-ITD. We will evaluate if FLT3-ITD expression and/or up-regulated protein products influence DC self-differentiation.(1<sup>st</sup> year)

**Aim 2:** To validate in a mouse model that mice immunized with DC/LV-FLT3-ITD will mount effective immunity against the oncogene and/or up-regulated protein products.(1<sup>st</sup>-2<sup>nd</sup> year)

**Aim 3:** To demonstrate therapeutic effects of DC/LV-FLT3-ITD vaccines against minimal residual disease recapitulated in a mouse transplantable FLT3-ITD leukemia model (32D) and evaluate immune cross-reactivity against different FLT3-ITD mutations.(2<sup>nd</sup>-3<sup>rd</sup> year)

**Group Members:** (either indicate the name(s) or the number of staff)



<p><b>Renata Stripecke</b> PhD (PI)</p> 	<p><b>Bala Sai Sundarasetty</b> MS (PhD -Rebirth)</p> 	<p><b>Mudita Pincha</b> MS (PhD Molec Med)</p> 	<p><b>Gustavo Salguero,</b> MD PhD (Post-doc)</p> 
<p><b>Meriam Nassiri</b> (BTA)</p> 	<p><b>Mareike Rickmann</b> (Strucmed)</p> 	<p><b>Andreas Schneider</b> (MTA)</p> 	<p><b>Sandra Kuhs</b> "Dr.rer.nat. "(Post-doc)</p> 

## Key References for project

- Koya, R. C., T. Kimura, et al. (2007). "Lentiviral vector-mediated autonomous differentiation of mouse bone marrow cells into immunologically potent dendritic cell vaccines." Mol Ther **15**(5): 971-80.
- Koya, R. C., J. S. Weber, et al. (2004). "Making dendritic cells from the inside out: lentiviral vector-mediated gene delivery of granulocyte-macrophage colony-stimulating factor and interleukin 4 into CD14<sup>+</sup> monocytes generates dendritic cells in vitro." Hum Gene Ther **15**(8): 733-48.
- Graf, C., F. Heidel, et al. (2007). "A neoepitope generated by an FLT3 internal tandem duplication (FLT3-ITD) is recognized by leukemia-reactive autologous CD8<sup>+</sup> T cells." Blood **109**(7): 2985-8.
- Scholl, S., S. Salzmann, et al. (2006). "Flt3-ITD mutations can generate leukaemia specific neoepitopes: potential role for immunotherapeutic approaches." Leuk Lymphoma **47**(2): 307-12.
- Breitenbuecher, F., S. Schnittger, et al. (2008). "Identification of a novel type of ITD mutations located in non-juxtamembrane domains of the FLT3 tyrosine kinase receptor." Blood.

## Own references (mainly 2004-2006):

1. Koya R., Kimura T., Ribas A., Rozengurt N., Lawson GW, Faure-Kumar E., Wang H-J, Herschmann H., Kasahara K. and Stripecke R (2007). Lentiviral vector-mediated autonomous differentiation of mouse bone marrow cells into immunologically potent dendritic cell vaccines. Mol Ther. 15(5):971-80
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3. Stripecke, R., Kasahara, N. Lentiviral and Retroviral Vector Systems. Cancer Drug Discovery and Development: Gene Therapy for Cancer. 2007. Dr. Kelly K. Hunt, ed.: 39-71
4. Cheng JC, Kinjo K, Judelson DR, Chang J, Wu WS, Schmid I, Shankar DB, Kasahara N, Stripecke R, Bhatia R, Landaw EM, Sakamoto KM (2008). CREB is a critical regulator of normal hematopoiesis and leukemogenesis. Blood 111(3):1182-92.
5. Köchling J, Prada J, Bahrami M, Stripecke R, Seeger K, Henze G, Wittig B, Schmidt M (2008). Anti-tumor effect of DNA-based vaccination and dSLIM immunomodulatory molecules in mice with Ph(+) acute lymphoblastic leukaemia. Vaccine 26;26(36):4669-4675.
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**Engagement in the MD/PhD program or HBRS:**

Supervisor: ✓ Name of student(s): E. Prurevdorj, K. Zscheppang  
Lecturer: ✓; PhD Kommission: ; No, not yet:

**Animal experiments involved:** ✓ yes  no

**Research focus:**

Our research focuses is on the role of neuregulin-1 (NRG) and the erbB receptor system in lung and brain development. In particular, we are interested in the roles of NRG and its receptor system in the pathogenesis of perinatal lung and brain disorders, and in protection against perinatal insults.

Neuregulin is a molecule with known complex actions in heart, lung, and brain development. It also plays a prominent role in breast cancer.

A few years ago we have found that NRG is part of the intercellular crosstalk between lung fibroblasts and pneumocytes, helping signal the onset of surfactant synthesis – a crucial component of lung maturity that is a survival factor in prematurity. Since 2006, we have published 7 papers on this and closely related perinatal pathomechanisms in the developing lung. Three of these were authored by previous and current MD/PhD students at HBRS. This line of research, led by Christiane Dammann, currently receives funding from the Deutsche Forschungsgemeinschaft (DFG) at MHH and the National Institutes of Health (NIH) at Tufts Medical Center, Boston, USA.

Over the past decade, we have also contributed to a paradigm shift in perinatal brain damage research. Until the early 1990s, energy failure was considered the major damage mechanism for brain damage in the preterm newborn. Since the mid-Nineties, we have published a series of papers that has convinced most members of the perinatal neuroscience community that antenatal subclinical infection and subsequent fetal inflammatory responses contribute to brain and retina damage in the immature newborn. This line of research is led by Olaf Dammann and is currently funded by the European Commission at MHH and by NIH at Tufts Medical Center, Boston, USA.

Recent pilot work done by Dr. Wolfgang Büter (MHH MD thesis '08) and Insa Hoffmann (MHH MD Thesis '09, pending) suggests that NRG might be a potent perinatal neuroprotector. In the proposed project, we suggest to pursue this line of research in the setting of systemic fetal inflammation.

**Specific project and methods applied:**

**Title:** Neuroprotection by neuregulin-1 in the setting of systemic inflammation.

**Aims:** **1.** To test the hypothesis that the brain damage associated with intrauterine exposure to LPS can be reduced by treatment with NRG. **2.** To compare the potentially protective effect of NRG treatment to the potentially protective effect of bone marrow stem cells (BMSC). **3.** To test the hypothesis that NRG signals its rescue from LPS-induced brain damage through ErbB4, one of the receptors for NRG-like growth factors.

**Funding:** European Commission (NEOBRAIN; LSHM-CT-2006-036534; NEUROBID; FP7-241778, pending)

Maternal infection and subsequent maternal and fetal inflammatory responses are important etiologic factors involved in the development of neonatal brain lesions. Excessive secretion of cytokines is part of the inflammatory responses at the systemic and brain levels, finally leading to astrogliosis, aberration of oligodendrocyte maturation, as well as necrotic and apoptotic cell death.

Transgenic ErbB4-deleted (HER4<sup>heart</sup><sup>-/-</sup>) mice, rescued from their lethal cardiac defects by expressing human ErbB4 (HER4) cDNA under the cardiac-specific  $\alpha$ -myosin heavy chain promoter (Tidcombe et al, PNAS 2003) will be used in these study. LPS (100g/kg), NRG (2 $\mu$ l/ml and 4 $\mu$ l/ml), or BMSC (2x10<sup>6</sup>) will be injected alone or in combination into the amniotic cavity at d17 of gestation in both dominant ErbB4 negative and wild-type animals as previously published. ErbB4 deletion potentiates (Behrens et al, 2008, abstract) and BMSC prevent most of the LPS-induced lung injury (Bokel et al, abstract, ATS 2008).

In this project, we will employ the effects of LPS, NRG and BMSCs on the perinatal brain. After intraperitoneal administration of LPS +/- NRG or LPS +/- BMSC, the mice will be sacrificed at different time points (E18, P5, P15 and P40). Both pro-inflammatory (e.g. IL-1 $\beta$ , TNF- $\alpha$ , IL-6) and anti-inflammatory (e.g. IL-10) cytokines and chemokines and other trophic factors (e.g. BDNF, NGF, IGF-1) will be determined by reverse transcription quantitative polymerase chain reaction. Brain injury will be examined in various histological brain sections by Tunel and cleaved caspase-3 (cell death), GFAP (astrogliosis), Iba1 and isolectin B4 (microgliosis), and O4, NG2, and MBP (oligodendrocytes and myelin) immunohistochemistry.

### **Group Members:**

NN, PhD student;

Katja Zscheppang, PhD student;

Andrea Liese, technician

Dietlinde von Mayersbach, technician

Dr. Wolfgang Büter, Post-Doc

Dr. Thilo Dörk-Bousset, Lab Director (MHH Gynecology)

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Prof. Martin Stangel, Vice Chair (MHH Neurology)

Prof. Pierre Gressens, Director (INSERM U-676)

Prof. Dr. Olaf Dammann, Director, (MHH Perinatal Neuroepidemiology, Tufts Newborn Med)

Prof. Dr. Christiane Dammann, Lab Director (MHH Pediatrics, Tufts Newborn Med)

### **Key References for project**

1. Corfas G, Roy K, Buxbaum JD. Neuregulin 1-erbB signaling and the molecular/cellular basis of schizophrenia. *Nat Neurosci* 2004;7:575-80.
2. Xu Z, Ford GD, Croslan DR, et al. Neuroprotection by neuregulin-1 following focal stroke is associated with the attenuation of ischemia-induced pro-inflammatory and stress gene expression. *Neurobiol Dis* 2005;19:461-70.
3. Mei L, Xiong WC. Neuregulin 1 in neural development, synaptic plasticity and schizophrenia. *Nat Rev Neurosci* 2008;9:437-52.
4. Birchmeier C. ErbB receptors and the development of the nervous system. *Exp Cell Res* 2009;315:611-8.

### **Own references (mainly 2004-2006):**

1. Dammann CE, Nielsen HC, Carraway KL, 3rd. Role of neuregulin-1 beta in the developing lung. *Am J Respir Crit Care Med* 2003;167:1711-6.
2. Dammann O, Leviton A. Inflammatory brain damage in preterm newborns - dry numbers, wet lab, and causal inference. *Early Hum Dev* 2004;79:1-15.
3. Zscheppang K, Korenbaum E, Bueter W, Ramadurai SM, Nielsen HC, Dammann CE. ErbB receptor dimerization, localization, and co-localization in mouse lung type II epithelial cells. *Pediatr Pulmonol* 2006;41:1205-12.
4. Dammann O, O'Shea TM. Cytokines and perinatal brain damage. *Clin Perinatol* 2008;35:643-63.
5. Dammann O, Bueter W, Leviton A, Gressens P, Dammann CE. Neuregulin-1: a potential endogenous protector in perinatal brain white matter damage. *Neonatology* 2008;93:182-7.

18.)

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**Engagement in the MD/PhD program or HBRs:**

Supervisor: yes Name of student(s): Reena Singh, Rannar Airik  
Lecturer: yes;

**Animal experiments involved:**  yes

**Research focus:**

Our research focuses on the genetic control mechanisms governing vertebrate development using the mouse as a model organism. More specifically, we are interested in gaining a better understanding of the cellular and molecular processes crucial in the development of mesodermally derived organs including the heart, kidney and ureter, and the inner ear. This work also aims to better understand congenital human diseases including valvular-septal defects of the heart, anomalies of the urinary tract and hearing loss by establishing mouse models for these disorders and deciphering the molecular changes associated with them. In the long run, this may provide means to reinitiate developmental programs for regenerative approaches in human diseases.

We are using all techniques of modern cell and molecular biology in addition to embryological manipulations in the mouse as well as transgenics and targeted gene disruption to gain information on the mode of action of genes in vivo and in vitro.

A major focus of our molecular work lies in the analysis of the role of transcription factors of the T-box (Tbx) gene family in organogenesis.

**Specific project and methods applied:**

**Title:** Genetic pathways governing the development of the ureteric mesenchyme in the mouse

**Aims:** In the proposed project the genetic pathways regulating the development of the ureteric mesenchyme shall be analyzed. In particular, the role of signaling pathways in the differentiation of the smooth muscle layer of the ureter shall be explored by genetic approaches in vivo.

**Funding:** Hausstelle

The urinary system is a multi-component entity, whose primary functions are the maintenance of body homeostasis by controlling the water and ionic balance of the blood and the excretion of excess water, solutes, and waste products. Rather than being a simple, passive tubular outlet of the pelvis, the ureter represents a pivotal connection between the upper (the kidneys) and lower urinary system (the bladder and the urethra). After filling the renal pelvis with urine, the upper portion of the ureter undergoes unidirectional peristaltic contractions, triggered by pacemaker cells, to propel the urine down to the bladder whilst preventing any reflux or efflux at the same time. The crucial importance of the ureter for renal function is dramatically reflected by acquired and inherited defects that interfere with the efficient removal of the urine from the renal pelvis. Any kind of anatomical or functional obstruction along the ureter or at its junctions will result in fluid pressure-mediated dilation of the tubular system proximal to the side of constriction (Hydroureter and hydronephrosis).

In contrast to other organs such as the kidney, our knowledge of the genetic control of ureterogenesis has been limited. However, recent findings suggest that formation of the ureter relies on a multi-step developmental program that is characterized by the interaction of different mesenchymal and epithelial cell lineages of the early metanephric field. Our own experiments have revealed the importance of the ureteric mesenchyme and the T-box transcription factor Tbx18 therein for this process. In Tbx18<sup>-/-</sup> mice, the ureteric mesenchyme disperses and remains undifferentiated leading to hydroureter

formation by functional obstruction. Here, we want to define the molecular pathways that confer aggregation and differentiation of the ureteric mesenchyme by genetic loss- and gain-of-function approaches in the mouse.

First, the functional significance of canonical Wnt signaling in survival and differentiation of ureteric mesenchyme shall be studied. This will involve conditional inactivation and conditional activation of the crucial intracellular signaling mediator b-catenin in the ureteric mesenchyme in vivo. The phenotypic consequences shall be studied and target genes shall be identified.

Second, the functional significance of non-canonical Wnt signaling in aggregation and differentiation of ureteric mesenchyme shall be studied. This will involve analysis of mouse lines mutant for Wnt5a and Wnt11. The phenotypic consequences shall be studied and target genes shall be identified.

Third, cell lines of ureteric mesenchyme shall be established after conditional activation of b-catenin therein.

Fourth, the relevance of other signaling pathways for smooth muscle differentiation shall be analyzed by pharmaceutical inhibition experiments in cultures of kidney rudiments.

### **Group Members:**

<2>, postdoc;

<10>, PhD student;

<2>, Diploma or Master student;

<2>, technician as of July 1st 2009

### **Key References for project**

1. Airik, R., Bussen, M., Singh, M.K., Petry, M. and Kispert, A. (2006). Tbx18 regulates the development of the ureteral mesenchyme. *J. Clin. Invest.* 116, 663-674.
2. Airik, R. and Kispert, A. (2007). Down the tube of obstructive nephropathies: the importance of tissue interactions in ureter development. *Kidney Int.* 72, 1459-1467.

### **Own references (mainly 2006-2009):**

#### **2009**

Christoffels, V.M., Grieskamp, T., Norden, J., Mommersteeg, M.T.M., Rudat, C., Kispert, A. (2009). Tbx18 and the fate of epicardial progenitors. *Nature* 458, E8-9.

Lüdtke, T., Christoffels, V.M., Petry, M., Kispert, A. (2009). Tbx3 Promotes Liver Bud Expansion During Mouse Development by Suppression of Cholangiocyte Differentiation. *Hepatology* 49, 969-978.

Kobayashi, K., Luo, M., Zhang, Y., Wilkes, D.C., Ge, G., Grieskamp, T., Yamada, C., Liu, T.C., Huan, G., Basson, C.T., Kispert, A., Greenspan, D.S., Sato, T.N. (2009). Secreted Frizzled Related Protein 2: a novel procollagen C-proteinase enhancer with a key role in myocardial infarction-associated fibrosis. *Nature Cell Biol.* 11, 46-55.

Wiese, C.\*, Grieskamp, T.\*, Airik, A., Mommersteeg, M.T.M., Gardiwal, A., de Gier-de Vries, C., Schuster-Gossler, K., Moorman, A.F.M., Kispert, A.\*, Christoffels, V.M.\* (2009). Formation of the Sinus Node Head and Differentiation of Sinus Node Myocardium Are Independently Regulated by Tbx18 and Tbx3. *Circ. Res.* 104, 388-397. \* equal contribution

#### **2008**

Winkler, M.E., Mauritz, C., Groos, S., Kispert, A., Menke, S., Hoffmann, A., Gruh, I., Schwanke, K., Haverich, A., Martin, U. (2008). Serum-free differentiation of murine embryonic stem cells into alveolar type II epithelial cells. *Cloning Stem Cells* 10, 49-64.

Potok, M.A., Cha, K.B., Hunt, A., Brinkmeier, M.L., Leitges, M., Kispert, A. and Camper, S.A. (2008). WNT signaling affects gene expression in the ventral diencephalon and pituitary gland growth. *Dev. Dyn.* 237, 1006-1020.

Bergmann, C., Fliegauf, M., Bruechle, N.O., Frank, V., Olbrich, H., Kirschner, J., Schermer, B., Schmedding, I., Kispert, A., Kränzlin, B., Nürnberg, G., Becker, C., Grimm, T., Girschick, G., Lynch, S.A., Kelehan, P., Senderek, J., Neuhaus, T.J., Stallmach, T., Zentgraf, H., Nürnberg, P., Gretz, N., Lo, C., Lienkamp, S., Schäfer, T., Walz, G., Benzing, T., Zerres, K., Omran, H. (2008). Loss of nephrocystin-3 function can cause embryonic lethality, Meckel-Gruber-like syndrome, situs inversus, and renal-hepatic-pancreatic dysplasia. *Am. J. Hum. Genet.* 82, 959-970.

- Trowe, O., Maier, H., Schweizer, M., Kispert, A. (2008). Deafness in mice lacking the T-box transcription factor Tbx18 in otic fibrocytes. *Development* 135, 1725-1734.
- Farin, H.F., Mansouri, A., Petry, M., Kispert, A. (2008). T-box Protein Tbx18 Interacts with the Paired Box Protein Pax3 in the Development of the Paraxial Mesoderm. *J. Biol. Chem.* 283, 25372-25380.
- Lausch, E., Hermanns, P., Farin, H.F., Alanay, Y., Unger, S., Nikkel, S., Steinwender, C., Scherer, G., Spranger, J., Zabel, B., Kispert, A., Superti-Furga, A. (2008). TBX15 mutations cause craniofacial dysmorphism, hypoplasia of scapula and pelvis, and short stature in Cousin syndrome. *Am. J. Hum. Genet.* 83, 649-655.

## 2007

- Kaelin, R.E., Kretz, M.P., Meyer, A.M., Kispert, A., Heppner, F.L. and Brändli, A.W. (2007). Paracrine and autocrine mechanisms of apelin signaling govern embryonic and tumor angiogenesis. *Dev. Biol.* 305, 599-614.
- Wittler, L., Shin, E.-h., Grote, P., Kispert, A., Beckers, A., Gossler, A., Werber, M., and Herrmann, B.G. (2007). Expression of Msn1 in the presomitic mesoderm is controlled by synergism of WNT signaling and Tbx6. *EMBO Reports* 8, 784-789.
- Farin, H.F., Bussen, M., Schmidt, M.K., Singh, M.K., Schuster-Gossler, K. and Kispert, A. (2007). Transcriptional repression by the T-box proteins Tbx18 and Tbx15 depends on Groucho corepressors. *J. Biol. Chem.* 282, 25748-25759.
- Reggiani, L., Raciti, D., Airik, R., Kispert, A. and Brändli, A.W. (2007). The prepattern transcription factor Irx3 directs nephron segment identity. *Genes Dev.* 21, 2358-2370.
- Andreou, A.M., Pauws, E., Jones, M.C., Singh, M.K., Bussen, M., Doudney, K., Moore, G.E., Kispert, A., Brosens, J.J. and Stanier, S. (2007). TBX22 missense mutations found in X-linked cleft palate (CPX) patients affect DNA binding, sumoylation and transcriptional repression. *Am. J. Hum. Gen.* 81, 700-712.
- Airik, R. and Kispert, A. (2007). Down the tube of obstructive nephropathies: the importance of tissue interactions in ureter development. *Kidney Int.* 72, 1459-1467.

## 2006

- Osafune, K., Takasato, M., Kispert, A., Asashima, M. and Nishinakamura, R. (2006). Identification of multipotent progenitors in the embryonic mouse kidney by a novel colony-forming assay. *Development* 133, 151-161.
- Sass, J.O., Mohr, V., Olbrich, H., Engelke, U., Horvath, J., Fliegau, M., Loges, N.T., Schweitzer-Krantz, S., Moebus, R., Weiler, P., Kispert, A., Superti-Furga, A., Wevers, R.A. and Omran, H. (2006). Mutations in ACY1, the gene encoding aminoacylase 1, cause a novel inborn error of metabolism. *Am. J. Hum. Genetics* 78, 401-409.
- Airik, R., Bussen, M., Singh, M.K., Petry, M. and Kispert, A. (2006). Tbx18 regulates the development of the ureteral mesenchyme. *J. Clin. Invest.* 116, 663-674.
- Jochheim-Richter, A., Rüdlich, U., Koczan, D., Hillemann, T., Tewes, S., Petry, M., Kispert, A., Deep Sharma, A., Attaran, F., Manns, M.P. and Ott, M. (2006). Gene expression analysis identifies novel genes participating in early murine liver development and adult liver regeneration. *Differentiation* 74, 167-173.
- Sayer, J.A., Otto, E.A., O'toole, J.F., Nurnberg, G., Kennedy, M.A., Becker, C., Hennies, H.C., Helou, J., Attanasio, M., Fausett, B.V., Utsch, B., Khanna, H., Liu, Y., Drummond, I., Kawakami, I., Kusakabe, T., Tsuda, M., Ma, L., Lee, H., Larson, R.G., Allen, S.J., Wilkinson, C.J., Nigg, E.A., Shou, C., Lillo, C., Williams, D.S., Hoppe, B., Kemper, M.J., Neuhaus, T., Parisi, M.A., Glass, I.A., Petry, M., Kispert, A., Gloy, J., Ganner, A., Walz, G., Zhu, X., Goldman, D., Nurnberg, P., Swaroop, A., Leroux, M.R. and Hildebrandt, F. (2006). The centrosomal protein nephrocystin-6 is mutated in Joubert syndrome and activates transcription factor ATF4. *Nat. Genet.* 38, 674-681.
- Franco, D., Meilhac, S.M., Christoffels, V.M., Kispert, A., Buckingham, M. and Kelly, R.G. (2006). Left and right ventricular contributions to the formation of the interventricular septum in the mouse heart. *Dev. Biol.* 294, 366-375.
- Christoffels, V.M., Mommersteeg, M.T.M., Trowe, M.-O., Prall, O.W.J., de Gier-de Vries, C., Soufan, A.T., Bussen, M., Schuster-Gossler, K., Harvey, R.P., Moorman, A.F.M. and Kispert, A. (2006). Formation of the venous pole of the heart from an Nkx2.5-negative precursor population requires Tbx18. *Circ. Res.* 98, 1555-1563.
- Barrionuevo, F., Taketo, M.M., Scherer, G. and Kispert, A. (2006). Sox9 is required for notochord maintenance in mice. *Dev. Biol.* 295, 128-140.
- Gerke, P., Benzing, T., Höhne, M., Kispert, A., Frotscher, M., Walz, G. and Kretz, O. (2006). Neuronal expression and interaction with the synaptic protein CASK suggest a role for Neph1 and Neph2 in synaptogenesis. *J. Comp. Neurol.* 498, 466-475.
- Kant, S., Schumacher, S., Singh, M.K., Kispert, A., Kotlyarov, A. and Gaestel, M. (2006). Characterization of the atypical MAP kinase ERK4 and its activation of the MAPK-activated protein kinase MK5. *J. Biol. Chem.* 281, 35511-35519.

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**Engagement in the MD/PhD program or HBRs:**

Supervisor: yes Name of student(s): Reena Singh, Rannar Airik  
Lecturer: yes;

**Animal experiments involved:**  yes

**Research focus:**

Our research focuses on the genetic control mechanisms governing vertebrate development using the mouse as a model organism. More specifically, we are interested in gaining a better understanding of the cellular and molecular processes crucial in the development of mesodermally derived organs including the heart, kidney and ureter, and the inner ear. This work also aims to better understand congenital human diseases including valvular-septal defects of the heart, anomalies of the urinary tract and hearing loss by establishing mouse models for these disorders and deciphering the molecular changes associated with them. In the long run, this may provide means to reinitiate developmental programs for regenerative approaches in human diseases.

We are using all techniques of modern cell and molecular biology in addition to embryological manipulations in the mouse as well as transgenics and targeted gene disruption to gain information on the mode of action of genes in vivo and in vitro.

A major focus of our molecular work lies in the analysis of the role of transcription factors of the T-box (Tbx) gene family in organogenesis.

**Specific project and methods applied:**

**Title:** Genetic pathways governing the development of the venous pole region of the murine heart

**Aims:** In the proposed project the role of Tbx18 in the development of the venous pole region of the murine heart shall be analyzed. This shall involve misexpression experiments in vivo, isolation of target genes and protein interaction partners.

**Funding:** Hausstelle

The systemic venous return of the heart (the cardiac inflow region) consists of multiple anatomical components including the proximal myocardial part of the right superior and inferior caval veins, the coronary sinus (the persisting left caval vein in the mouse), and the sinus venarum. Developmental disorders of the heart, which include malformations of the pulmonary and systemic venous returns represent the most common human birth defects. In addition, several specific components of the venous return including the sinoatrial node, the pacemaker of the heart, are found to be the origin of arrhythmias. Despite this importance of the cardiac venous pole in development and disease little is known on the genetic and cellular programs that govern its development.

Using genetic lineage analysis, we have recently shown that the myocardial sinus horns and their precursor form only after heart looping from a novel mesenchymal precursor population expressing the T-box transcription factor gene Tbx18. We have demonstrated that Tbx18 is essential for the formation of the sinus horns from the mesenchyme of the pericardial wall and for their myocardial differentiation. In addition, the sinoatrial node is severely reduced in size in Tbx18-deficient embryos.

In this project, we wish to continue our analysis of the molecular and cellular function of Tbx18 in the development of the inflow region of the heart by further analyzing the molecular circuitry operated by Tbx18.

First, the function of the T-box transcription factor Tbx18 in inflow tract development shall be

analyzed by misexpression experiments in vivo. We have developed a genetic system that allows the expression of wildtype Tbx18 protein as well as a dominant negative form of Tbx18 in the different heart progenitor pools contributing to cardiac development. The consequence of this misexpression for cardiac function and architecture shall be analyzed in detail.

Second, we have recently performed a microarray screen of Gfp-sorted Tbx18-positive and Tbx18-negative cells from the inflow region of the embryonic heart. This screen shall be validated by in situ hybridization analysis of candidate genes in wildtype and Tbx18-deficient embryos to find possible downstream mediators of Tbx18 function and more generally genes with a restricted expression in the cardiac inflow region. This shall give new insights into the genetic components involved in formation and myocardialization of the venous pole of the heart. Genes of interest will be functionally analyzed by conditional gene targeting. We have recently established a cre Knock-in line of Tbx18 that allows the functional analysis of genes in the developing caval veins.

Third, protein interaction partners of Tbx18 protein shall be identified by TAPtag technology in vivo. For this, we have initiated the generation of mice for the conditional expression of a Tbx18TAP-tagged protein that will enable us to screen for direct protein interaction partners of Tbx18 in extracts from mouse tissues expressing this fusion protein.

### **Group Members:**

<2>, postdoc;<10>, PhD student;<2>, Diploma or Master student;<2>, technician as of July 1st 2009

### **Key References for project**

1. Soufan, A.T., Bussen, M., Schuster-Gossler, K., Harvey, R.P., Moorman, A.F.M. and Kispert, A. (2006). Formation of the venous pole of the heart from an Nkx2.5-negative precursor population requires Tbx18. *Circ. Res.* 98, 1555-1563.
2. Farin, H.F., Bussen, M., Schmidt, M.K., Singh, M.K., Schuster-Gossler, K. and Kispert, A. (2007). Transcriptional repression by the T-box proteins Tbx18 and Tbx15 depends on Groucho corepressors. *J. Biol. Chem.* 282, 25748-25759.
3. Mommersteeg MT, Hoogaars WM, Prall OW, de Gier-de Vries C, Wiese C, Clout DE, Papaioannou VE, Brown NA, Harvey RP, Moorman AF, Christoffels VM (2007). Molecular pathway for the localized formation of the sinoatrial node. *Circ Res* 100, 354-62.
4. Blaschke RJ, Hahurij ND, Kuijper S, Just S, Wisse LJ, Deissler K, Maxelon T, Anastassiadis K, Spitzer J, Hardt SE, Schöler H, Feitsma H, Rottbauer W, Blum M, Meijlink F, Rappold G, Gittenberger-de Groot AC (2008). Targeted mutation reveals essential functions of the homeodomain transcription factor Shox2 in sinoatrial and pacemaking development. *Circulation* 115, 1830-8.
5. Wiese, C.\*, Grieskamp, T.\*, Airik, A., Mommersteeg, M.T.M., Gardiwal, A., de Gier-de Vries, C., Schuster-Gossler, K., Moorman, A.F.M., Kispert, A.\*, Christoffels, V.M.\* (2009). Formation of the Sinus Node Head and Differentiation of Sinus Node Myocardium Are Independently Regulated by Tbx18 and Tbx3. *Circ. Res.* 104, 388-397. \* equal contribution

### **Own references (mainly 2006-2009):**

#### **2009**

- Christoffels, V.M., Grieskamp, T., Norden, J., Mommersteeg, M.T.M., Rudat, C., Kispert, A. (2009). Tbx18 and the fate of epicardial progenitors. *Nature* 458, E8-9.
- Lüdtke, T., Christoffels, V.M., Petry, M., Kispert, A. (2009). Tbx3 Promotes Liver Bud Expansion During Mouse Development by Suppression of Cholangiocyte Differentiation. *Hepatology* 49, 969-978.
- Kobayashi, K., Luo, M., Zhang, Y., Wilkes, D.C., Ge, G., Grieskamp, T., Yamada, C., Liu, T.C., Huan, G., Basson, C.T., Kispert, A., Greenspan, D.S., Sato, T.N. (2009). Secreted Frizzled Related Protein 2: a novel procollagen C-proteinase enhancer with a key role in myocardial infarction-associated fibrosis. *Nature Cell Biol.* 11, 46-55.
- Wiese, C.\*, Grieskamp, T.\*, Airik, A., Mommersteeg, M.T.M., Gardiwal, A., de Gier-de Vries, C., Schuster-Gossler, K., Moorman, A.F.M., Kispert, A.\*, Christoffels, V.M.\* (2009). Formation of the Sinus Node Head and Differentiation of Sinus Node Myocardium Are Independently Regulated by Tbx18 and Tbx3. *Circ. Res.* 104, 388-397. \* equal contribution

## 2008

- Winkler, M.E., Mauritz, C., Groos, S., Kispert, A., Menke, S., Hoffmann, A., Gruh, I., Schwanke, K., Haverich, A., Martin, U. (2008). Serum-free differentiation of murine embryonic stem cells into alveolar type II epithelial cells. *Cloning Stem Cells* 10, 49-64.
- Potok, M.A., Cha, K.B., Hunt, A., Brinkmeier, M.L., Leitges, M., Kispert, A. and Camper, S.A. (2008). WNT signaling affects gene expression in the ventral diencephalon and pituitary gland growth. *Dev. Dyn.* 237, 1006-1020.
- Bergmann, C., Fliegau, M., Bruechle, N.O., Frank, V., Olbrich, H., Kirschner, J., Schermer, B., Schmedding, I., Kispert, A., Kränzlin, B., Nürnberg, G., Becker, C., Grimm, T., Girschick, G., Lynch, S.A., Kelehan, P., Senderek, J., Neuhaus, T.J., Stallmach, T., Zentgraf, H., Nürnberg, P., Gretz, N., Lo, C., Lienkamp, S., Schäfer, T., Walz, G., Benzing, T., Zerres, K., Omran, H. (2008). Loss of nephrocystin-3 function can cause embryonic lethality, Meckel-Gruber-like syndrome, situs inversus, and renal-hepatic-pancreatic dysplasia. *Am. J. Hum. Genet.* 82, 959-970.
- Trowe, O., Maier, H., Schweizer, M., Kispert, A. (2008). Deafness in mice lacking the T-box transcription factor Tbx18 in otic fibrocytes. *Development* 135, 1725-1734.
- Farin, H.F., Mansouri, A., Petry, M., Kispert, A. (2008). T-box Protein Tbx18 Interacts with the Paired Box Protein Pax3 in the Development of the Paraxial Mesoderm. *J. Biol. Chem.* 283, 25372-25380.
- Lausch, E., Hermanns, P., Farin, H.F., Alanay, Y., Unger, S., Nikkel, S., Steinwender, C., Scherer, G., Spranger, J., Zabel, B., Kispert, A., Superti-Furga, A. (2008). TBX15 mutations cause craniofacial dysmorphism, hypoplasia of scapula and pelvis, and short stature in Cousin syndrome. *Am. J. Hum. Genet.* 83, 649-655.

## 2007

- Kaelin, R.E., Kretz, M.P., Meyer, A.M., Kispert, A., Heppner, F.L. and Brändli, A.W. (2007). Paracrine and autocrine mechanisms of apelin signaling govern embryonic and tumor angiogenesis. *Dev. Biol.* 305, 599-614.
- Wittler, L., Shin, E.-h., Grote, P., Kispert, A., Beckers, A., Gossler, A., Werber, M., and Herrmann, B.G. (2007). Expression of Msn1 in the presomitic mesoderm is controlled by synergism of WNT signaling and Tbx6. *EMBO Reports* 8, 784-789.
- Farin, H.F., Bussen, M., Schmidt, M.K., Singh, M.K., Schuster-Gossler, K. and Kispert, A. (2007). Transcriptional repression by the T-box proteins Tbx18 and Tbx15 depends on Groucho corepressors. *J. Biol. Chem.* 282, 25748-25759.
- Reggiani, L., Raciti, D., Airik, R., Kispert, A. and Brändli, A.W. (2007). The prepattern transcription factor Irx3 directs nephron segment identity. *Genes Dev.* 21, 2358-2370.
- Andreou, A.M., Pauws, E., Jones, M.C., Singh, M.K., Bussen, M., Doudney, K., Moore, G.E., Kispert, A., Brosens, J.J. and Stanier, S. (2007). TBX22 missense mutations found in X-linked cleft palate (CPX) patients affect DNA binding, sumoylation and transcriptional repression. *Am. J. Hum. Gen.* 81, 700-712.
- Airik, R. and Kispert, A. (2007). Down the tube of obstructive nephropathies: the importance of tissue interactions in ureter development. *Kidney Int.* 72, 1459-1467.

## 2006

- Osafune, K., Takasato, M., Kispert, A., Asashima, M. and Nishinakamura, R. (2006). Identification of multipotent progenitors in the embryonic mouse kidney by a novel colony-forming assay. *Development* 133, 151-161.
- Sass, J.O., Mohr, V., Olbrich, H., Engelke, U., Horvath, J., Fliegau, M., Loges, N.T., Schweitzer-Krantz, S., Moebus, R., Weiler, P., Kispert, A., Superti-Furga, A., Wevers, R.A. and Omran, H. (2006). Mutations in ACY1, the gene encoding aminoacylase 1, cause a novel inborn error of metabolism. *Am. J. Hum. Genetics* 78, 401-409.
- Airik, R., Bussen, M., Singh, M.K., Petry, M. and Kispert, A. (2006). Tbx18 regulates the development of the ureteral mesenchyme. *J. Clin. Invest.* 116, 663-674.
- Jochheim-Richter, A., Rüdlich, U., Koczan, D., Hillemann, T., Tewes, S., Petry, M., Kispert, A., Deep Sharma, A., Attaran, F., Manns, M.P. and Ott, M. (2006). Gene expression analysis identifies novel genes participating in early murine liver development and adult liver regeneration. *Differentiation* 74, 167-173.
- Sayer, J.A., Otto, E.A., O'toole, J.F., Nürnberg, G., Kennedy, M.A., Becker, C., Hennies, H.C., Helou, J., Attanasio, M., Fausett, B.V., Utsch, B., Khanna, H., Liu, Y., Drummond, I., Kawakami, I., Kusakabe, T., Tsuda, M., Ma, L., Lee, H., Larson, R.G., Allen, S.J., Wilkinson, C.J., Nigg, E.A., Shou, C., Lillo, C., Williams, D.S., Hoppe, B., Kemper, M.J., Neuhaus, T., Parisi, M.A., Glass, I.A., Petry, M., Kispert, A., Gloy, J., Ganner, A., Walz, G., Zhu, X., Goldman, D., Nürnberg, P., Swaroop, A., Leroux, M.R. and Hildebrandt, F. (2006). The centrosomal protein nephrocystin-6 is mutated in Joubert syndrome and activates transcription factor ATF4. *Nat. Genet.* 38, 674-681.

- Franco, D., Meilhac, S.M., Christoffels, V.M., Kispert, A., Buckingham, M. and Kelly, R.G. (2006). Left and right ventricular contributions to the formation of the interventricular septum in the mouse heart. *Dev. Biol.* 294, 366-375.
- Christoffels, V.M., Mommersteeg, M.T.M., Trowe, M.-O., Prall, O.W.J., de Gier-de Vries, C., Soufan, A.T., Bussen, M., Schuster-Gossler, K., Harvey, R.P., Moorman, A.F.M. and Kispert, A. (2006). Formation of the venous pole of the heart from an Nkx2.5-negative precursor population requires Tbx18. *Circ. Res.* 98, 1555-1563.
- Barrionuevo, F., Taketo, M.M., Scherer, G. and Kispert, A. (2006). Sox9 is required for notochord maintenance in mice. *Dev. Biol.* 295, 128-140.
- Gerke, P., Benzing, T., Höhne, M., Kispert, A., Frotscher, M., Walz, G. and Kretz, O. (2006). Neuronal expression and interaction with the synaptic protein CASK suggest a role for Neph1 and Neph2 in synaptogenesis. *J. Comp. Neurol.* 498, 466-475.
- Kant, S., Schumacher, S., Singh, M.K., Kispert, A., Kotlyarov, A. and Gaestel, M. (2006). Characterization of the atypical MAP kinase ERK4 and its activation of the MAPK-activated protein kinase MK5. *J. Biol. Chem.* 281, 35511-35519.

## 20.)

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### Engagement in the MD/PhD program or HBRS:

Supervisor: yes Name of student(s): Reena Singh, Rannar Airik  
 Lecturer: yes;

**Animal experiments involved:**  yes

### Research focus:

Our research focuses on the genetic control mechanisms governing vertebrate development using the mouse as a model organism. More specifically, we are interested in gaining a better understanding of the cellular and molecular processes crucial in the development of mesodermally derived organs including the heart, kidney and ureter, and the inner ear. This work also aims to better understand congenital human diseases including valvular-septal defects of the heart, anomalies of the urinary tract and hearing loss by establishing mouse models for these disorders and deciphering the molecular changes associated with them. In the long run, this may provide means to reinstate developmental programs for regenerative approaches in human diseases.

We are using all techniques of modern cell and molecular biology in addition to embryological manipulations in the mouse as well as transgenics and targeted gene disruption to gain information on the mode of action of genes in vivo and in vitro.

A major focus of our molecular work lies in the analysis of the role of transcription factors of the T-box (Tbx) gene family in organogenesis.

### Specific project and methods applied:

**Title:** Mobilization and differentiation of epicardial cells in the murine heart

**Aims:** In the proposed project the role of Tbx18 in epicardial development shall be analyzed. In addition, embryonic signaling pathways shall be reactivated in adult epicardium to analyze the regenerative potential of these cells.

**Funding:** DFG

We have recently generated a mouse mutant for Tbx18 by gene targeting in ES cells. Tbx18 The function of the vertebrate multi-chambered heart critically depends on a constant blood supply by the coronary vasculature. Coronary vasculature derives from an extracardiac source, the (pro-) epicardium, during embryogenesis. Its developmental program can be broken up into distinct steps. Induction of epicardial cells from lateral plate mesoderm, specification of these cells, migration of proepicardial cells onto the myocardium, formation of a single-layered epithelium, epithelial-mesenchymal transition, invasion of the subepicardial space, differentiation into various cell types including smooth muscle cells, fibroblasts and endothelial cells, and coronary vessel assembly. In the adult mammalian heart epicardial cells seem to have lost the capability to provide a cellular contribution to the underlying myocardium and the coronary vasculature hampering the regenerative potential of the myocardium after injury. Elucidation of the genetic control mechanisms that govern epicardial mobilization and differentiation in development may provide a molecular handle to reinitiate these processes in the adult heart during to promote myocardial regeneration after infarct.

We have recently identified genes with specific expression in the developing and mature epicardium. In this project we want to analyze the epicardial function of these genes on one hand and use the specific expression of these genes to develop tools for tracing and manipulating epicardial cells in vivo.

First, the function of the T-box transcription factor Tbx18 shall be analyzed in epicardial development by misexpression experiments in vivo. We have developed a genetic system that allows the expression of wildtype Tbx18 protein as well as a dominant negative form of Tbx18 in epicardial and epicardially derived cells. The consequence of this misexpression on epicardial function and architecture shall be analyzed in detail.

Second, based on the specific expression of a gene in the adult epicardium a genetic system shall be established in the mouse to trace epicardial cells in the adult heart. This CreERT2 knock-in allele shall also be used to reactivate embryonic signaling pathways in the adult epicardium by conditional gene activation strategies. Along this line a couple of transgenic lines shall be developed that allow the reactivation of developmental epicardial genes.

Therefore, we will try to lay ground in this project for a more thorough understanding of epicardial and coronary vasculature development in the mouse embryo and explore the possibilities to reactivate embryonic programs in the adult heart as a means for cellular therapies.

### **Group Members:**

<2>, postdoc;<10>, PhD student;<2>, Diploma or Master student;<2>, technician as of July 1st 2009

### **Key References for project**

1. Manner J, Perez-Pomares JM, Macias D, Munoz-Chapuli R. The origin, formation and developmental significance of the epicardium: a review. *Cells Tissues Organs*. 2001;169:89-103.
2. Winter EM, Gittenberger-de Groot AC. Epicardium-derived cells in cardiogenesis and cardiac regeneration. *Cell Mol Life Sci*. 2007;64:692-703.
3. Limana F, Zacheo A, Mocini D, Mangoni A, Borsellino G, Diamantini A, De Mori R, Battistini L, Vigna E, Santini M, Loiaconi V, Pompilio G, Germani A, Capogrossi MC. Identification of myocardial and vascular precursor cells in human and mouse epicardium. *Circ Res*. 2007;101:1255-1265.
4. Lepilina A, Coon AN, Kikuchi K, Holdway JE, Roberts RW, Burns CG, Poss KD. A dynamic epicardial injury response supports progenitor cell activity during zebrafish heart regeneration. *Cell*. 2006;127:607-619.
5. Christoffels, V.M., Grieskamp, T., Norden, J., Mommersteeg, M.T.M., Rudat, C., Kispert, A. (2009). Tbx18 and the fate of epicardial progenitors. *Nature* 458, E8-9.

### **Own references (mainly 2006-2009):**

#### **2009**

Christoffels, V.M., Grieskamp, T., Norden, J., Mommersteeg, M.T.M., Rudat, C., Kispert, A. (2009). Tbx18 and the fate of epicardial progenitors. *Nature* 458, E8-9.

- Lüdtke, T., Christoffels, V.M., Petry, M., Kispert, A. (2009). Tbx3 Promotes Liver Bud Expansion During Mouse Development by Suppression of Cholangiocyte Differentiation. *Hepatology* 49, 969-978.
- Kobayashi, K., Luo, M., Zhang, Y., Wilkes, D.C., Ge, G., Grieskamp, T., Yamada, C., Liu, T.C., Huan, G., Basson, C.T., Kispert, A., Greenspan, D.S., Sato, T.N. (2009). Secreted Frizzled Related Protein 2: a novel procollagen C-proteinase enhancer with a key role in myocardial infarction-associated fibrosis. *Nature Cell Biol.* 11, 46-55.
- Wiese, C.\*, Grieskamp, T.\*, Airik, A., Mommersteeg, M.T.M., Gardiwal, A., de Gier-de Vries, C., Schuster-Gossler, K., Moorman, A.F.M., Kispert, A.\*, Christoffels, V.M.\* (2009). Formation of the Sinus Node Head and Differentiation of Sinus Node Myocardium Are Independently Regulated by Tbx18 and Tbx3. *Circ. Res.* 104, 388-397. \* equal contribution

## 2008

- Winkler, M.E., Mauritz, C., Groos, S., Kispert, A., Menke, S., Hoffmann, A., Gruh, I., Schwanke, K., Haverich, A., Martin, U. (2008). Serum-free differentiation of murine embryonic stem cells into alveolar type II epithelial cells. *Cloning Stem Cells* 10, 49-64.
- Potok, M.A., Cha, K.B., Hunt, A., Brinkmeier, M.L., Leitges, M., Kispert, A. and Camper, S.A. (2008). WNT signaling affects gene expression in the ventral diencephalon and pituitary gland growth. *Dev. Dyn.* 237, 1006-1020.
- Bergmann, C., Fliegau, M., Bruechle, N.O., Frank, V., Olbrich, H., Kirschner, J., Schermer, B., Schmedding, I., Kispert, A., Kränzlin, B., Nürnberg, G., Becker, C., Grimm, T., Girschick, G., Lynch, S.A., Kelehan, P., Senderek, J., Neuhaus, T.J., Stallmach, T., Zentgraf, H., Nürnberg, P., Gretz, N., Lo, C., Lienkamp, S., Schäfer, T., Walz, G., Benzing, T., Zerres, K., Omran, H. (2008). Loss of nephrocystin-3 function can cause embryonic lethality, Meckel-Gruber-like syndrome, situs inversus, and renal-hepatic-pancreatic dysplasia. *Am. J. Hum. Genet.* 82, 959-970.
- Trowe, O., Maier, H., Schweizer, M., Kispert, A. (2008). Deafness in mice lacking the T-box transcription factor Tbx18 in otic fibrocytes. *Development* 135, 1725-1734.
- Farin, H.F., Mansouri, A., Petry, M., Kispert, A. (2008). T-box Protein Tbx18 Interacts with the Paired Box Protein Pax3 in the Development of the Paraxial Mesoderm. *J. Biol. Chem.* 283, 25372-25380.
- Lausch, E., Hermanns, P., Farin, H.F., Alanay, Y., Unger, S., Nikkel, S., Steinwender, C., Scherer, G., Spranger, J., Zabel, B., Kispert, A., Superti-Furga, A. (2008). TBX15 mutations cause craniofacial dysmorphism, hypoplasia of scapula and pelvis, and short stature in Cousin syndrome. *Am. J. Hum. Genet.* 83, 649-655.

## 2007

- Kaelin, R.E., Kretz, M.P., Meyer, A.M., Kispert, A., Heppner, F.L. and Brändli, A.W. (2007). Paracrine and autocrine mechanisms of apelin signaling govern embryonic and tumor angiogenesis. *Dev. Biol.* 305, 599-614.
- Wittler, L., Shin, E.-h., Grote, P., Kispert, A., Beckers, A., Gossler, A., Werber, M., and Herrmann, B.G. (2007). Expression of Msn1 in the presomitic mesoderm is controlled by synergism of WNT signaling and Tbx6. *EMBO Reports* 8, 784-789.
- Farin, H.F., Bussen, M., Schmidt, M.K., Singh, M.K., Schuster-Gossler, K. and Kispert, A. (2007). Transcriptional repression by the T-box proteins Tbx18 and Tbx15 depends on Groucho corepressors. *J. Biol. Chem.* 282, 25748-25759.
- Reggiani, L., Raciti, D., Airik, R., Kispert, A. and Brändli, A.W. (2007). The prepattern transcription factor Irx3 directs nephron segment identity. *Genes Dev.* 21, 2358-2370.
- Andreou, A.M., Pauws, E., Jones, M.C., Singh, M.K., Bussen, M., Doudney, K., Moore, G.E., Kispert, A., Brosens, J.J. and Stanier, S. (2007). TBX22 missense mutations found in X-linked cleft palate (CPX) patients affect DNA binding, sumoylation and transcriptional repression. *Am. J. Hum. Gen.* 81, 700-712.
- Airik, R. and Kispert, A. (2007). Down the tube of obstructive nephropathies: the importance of tissue interactions in ureter development. *Kidney Int.* 72, 1459-1467.

## 2006

- Osafune, K., Takasato, M., Kispert, A., Asashima, M. and Nishinakamura, R. (2006). Identification of multipotent progenitors in the embryonic mouse kidney by a novel colony-forming assay. *Development* 133, 151-161.
- Sass, J.O., Mohr, V., Olbrich, H., Engelke, U., Horvath, J., Fliegau, M., Loges, N.T., Schweitzer-Krantz, S., Moebus, R., Weiler, P., Kispert, A., Superti-Furga, A., Wevers, R.A. and Omran, H. (2006). Mutations in ACY1, the gene encoding aminoacylase 1, cause a novel inborn error of metabolism. *Am. J. Hum. Genetics* 78, 401-409.

- Airik, R., Bussen, M., Singh, M.K., Petry, M. and Kispert, A. (2006). Tbx18 regulates the development of the ureteral mesenchyme. *J. Clin. Invest.* 116, 663-674.
- Jochheim-Richter, A., Rüdlich, U., Koczan, D., Hillemann, T., Tewes, S., Petry, M., Kispert, A., Deep Sharma, A., Attaran, F., Manns, M.P. and Ott, M. (2006). Gene expression analysis identifies novel genes participating in early murine liver development and adult liver regeneration. *Differentiation* 74, 167-173.
- Sayer, J.A., Otto, E.A., O'toole, J.F., Nurnberg, G., Kennedy, M.A., Becker, C., Hennies, H.C., Helou, J., Attanasio, M., Fausett, B.V., Utsch, B., Khanna, H., Liu, Y., Drummond, I., Kawakami, I., Kusakabe, T., Tsuda, M., Ma, L., Lee, H., Larson, R.G., Allen, S.J., Wilkinson, C.J., Nigg, E.A., Shou, C., Lillo, C., Williams, D.S., Hoppe, B., Kemper, M.J., Neuhaus, T., Parisi, M.A., Glass, I.A., Petry, M., Kispert, A., Gloy, J., Ganner, A., Walz, G., Zhu, X., Goldman, D., Nurnberg, P., Swaroop, A., Leroux, M.R. and Hildebrandt, F. (2006). The centrosomal protein nephrocystin-6 is mutated in Joubert syndrome and activates transcription factor ATF4. *Nat. Genet.* 38, 674-681.
- Franco, D., Meilhac, S.M., Christoffels, V.M., Kispert, A., Buckingham, M. and Kelly, R.G. (2006). Left and right ventricular contributions to the formation of the interventricular septum in the mouse heart. *Dev. Biol.* 294, 366-375.
- Christoffels, V.M., Mommersteeg, M.T.M., Trowe, M.-O., Prall, O.W.J., de Gier-de Vries, C., Soufan, A.T., Bussen, M., Schuster-Gossler, K., Harvey, R.P., Moorman, A.F.M. and Kispert, A. (2006). Formation of the venous pole of the heart from an Nkx2.5-negative precursor population requires Tbx18. *Circ. Res.* 98, 1555-1563.
- Barrionuevo, F., Taketo, M.M., Scherer, G. and Kispert, A. (2006). Sox9 is required for notochord maintenance in mice. *Dev. Biol.* 295, 128-140.
- Gerke, P., Benzing, T., Höhne, M., Kispert, A., Frotscher, M., Walz, G. and Kretz, O. (2006). Neuronal expression and interaction with the synaptic protein CASK suggest a role for Neph1 and Neph2 in synaptogenesis. *J. Comp. Neurol.* 498, 466-475.
- Kant, S., Schumacher, S., Singh, M.K., Kispert, A., Kotlyarov, A. and Gaestel, M. (2006). Characterization of the atypical MAP kinase ERK4 and its activation of the MAPK-activated protein kinase MK5. *J. Biol. Chem.* 281, 35511-35519.

21.)

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Supervisor: X Name of student(s): Manoj Menon  
Lecturer: X; PhD Kommission: X; No, not yet:

**Animal experiments involved:**  yes ? no

**Research focus:**

Our research focuses on the MAP kinase signal transduction pathway downstream to p38. We generated gene targeting mice lacking p38 downstream kinases MK2(1) and MK3 (2). Animals with genetic disruption of these kinases produce significant lower amount of TNF $\alpha$ , IL6, and IFN $\gamma$ , making MKs an emerging target for anti-inflammatory drugs (reviewed in (3)). Recently, using genetic approach, we could reveal tristetraprolin (AU-rich binding protein) as an essential component of cytokine regulation by p38/MKs axis (4). However molecular mechanism of such regulation remains elusive.

**Specific project and methods applied:**

**Title:** Posttranscriptional regulation of cytokine expression

**Aims:** In the proposed project the function of MK2 and MK3 mediated phosphorylation of tristetraprolin should be investigated

**Funding:** DFG

The zinc finger protein tristetraprolin (TTP), also known as Nup 475, TIS11, G0S24, or ZFP36, mediates the instability of mRNAs that contain adenylate/uridylylate- rich elements (AREs) in their 3' untranslated region (3'UTR). AREs that contain the pentamer AUUUA are wide spread, and a computational estimation suggests that as much as 8% of human genes contain functional AREs, making a total of 4000 ARE-containing transcripts. Posttranslational mechanism of regulation TTP-deficient mice have a systemic autoimmune inflammatory syndrome with severe arthritis. The syndrome is predominantly due to excess circulating TNF, resulting from the increased stability of the TNF mRNA and subsequent higher rates of secretion of the cytokine controls its own expression by binding to the 3'UTR of its own mRNA, which also contains AREs. Following stimulation of macrophages by LPS, TTP is increasingly detected in multiple, differentially phosphorylated forms. Recently, links between TTP and the p38 mitogen-activated protein kinase (MAPK) pathway have been established. The p38 MAPK pathway is activated by stress and inflammatory stimuli and plays a major role in the regulation of the stability and/or translation of ARE containing transcripts. MAPK-activated protein kinase 2 (MK2) is one of several downstream targets of p38 The generation and analysis of MK2-deficient mice demonstrated that MK2 is the major kinase downstream of p38 responsible for posttranscriptional regulation of cytokine biosynthesis. The effect of MK2 on TNF biosynthesis is directly dependent on the ARE in the 3'UTR of TNF mRNA. TTP is a direct substrate of p38 and MK2 and the mouse protein is phosphorylated by MK2 at two major sites: serine 52 (S52) and S178. MK2/TTP double knockout bone marrow-derived macrophages (BMDM) produce TNF mRNA and protein

levels comparable to those of TTP knockout cells, indicating that TTP is genetically downstream of MK2 in the regulation of TNF mRNA stability and translation. It seems that phosphorylation by MK2 leads to TTP inhibition at least partly by reducing its affinity to AU-rich elements. The p38 MAPK pathway also regulates the subcellular localization and stability of TTP protein. A p38 MAPK inhibitor causes rapid dephosphorylation of TTP by an unknown phosphatase, degradation by the 20S/26S proteasome or relocation from the cytoplasm to the nucleus. . Hence, continuous activity of the p38 MAPK pathway is required to maintain the phosphorylation status, cytoplasmic localization, and stability of TTP protein. Other protein factors bind to ARE as well, such as HuR, AUF1, AUF2, FBP1, FBP2 (KSRP), TIA-1, and TIAR. These factors also regulate the stability and translation of ARE containing mRNAs. However all these proteins possess no enzymatic (nuclease) activity and have link ARE-containing mRNAs to degradation machinery or sequester mRNAs, protecting them from translation. These interactions remain elusive until now..

Using the cells derived from MK2 and MK3 knockout mice we will study the mechanism of posttranscriptional regulation of cytokine production. TTP knockout animals are housed in our animal facility in the frame of collaboration with P. Blackshear (Montreal). MEFs and macrophages (primary as well as immortalized cell lines) from TTP deficient animals should be established and either used in the project.

This study will include Y2H screening for tristetraprolin interacting proteins, analysis of tristetraprolin phosphorylation, and different reporter systems to elucidate the impact of this phosphorylation in regulation of translation and stability of cytokine mRNAs.

Interaction candidates, found in Y2H screening, should be analyzed in RNA EMSA assay, using already established EMSA with IR-Dye labeled ARE containing RNA. Interactors may cause supershift in TTP-ARE EMSA or affect TTP-ARE interaction. Overexpression of candidate proteins in macrophage cell lines or siRNA experiments with subsequent TNF quantification after LPS challenge should confirm the biological significance of found interactions.

Effect of TTP, phosphomimicking TTP mutants as well as TTP interacting proteins on the translation of ARE containing mRNAs will be studied in vitro using in cell free transcription-translation system and in vivo, using reporter assay with bicystronic vector, containing Renilla and Firefly luciferase, subcloned with different AREs or without such regulatory elements. Cell lines, derived from WT, MK2 knockout or TTP knockout animals could be used in such reporter experiments to understand the relative impact of these genes on ARE-mediated regulation. Usage of translational reporter in combination with up- or downregulation of proteins of interest provide a good tool for investigation of posttranscriptional regulation of cytokine expression. In situ hybridization and polysome profile are further tools, we will apply to study the turnover of ARE containing mRNAs.

### **Key References for project**

1. Kotlyarov, A., Neininger, A., Schubert, C., Eckert, R., Birchmeier, C., Volk, H. D., and Gaestel, M. (1999) *Nature cell biology* **1**(2), 94-97
2. Ronkina, N., Kotlyarov, A., Dittrich-Breiholz, O., Kracht, M., Hitti, E., Milarski, K., Askew, R., Marusic, S., Lin, L. L., Gaestel, M., and Telliez, J. B. (2007) *Molecular and cellular biology* **27**(1), 170-181
3. Gaestel, M. (2006) *Nature reviews* **7**(2), 120-130
4. Hitti, E., Iakovleva, T., Brook, M., Deppenmeier, S., Gruber, A. D., Radzioch, D., Clark, A. R., Blackshear, P. J., Kotlyarov, A., and Gaestel, M. (2006) *Molecular and cellular biology* **26**(6), 2399-2407

22.)

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Supervisor:  Name of student(s):

Lecturer: ; PhD Kommission: ; No, not yet:

**Animal experiments involved:**  yes  no

**Introduction:**

Asthma is a chronic inflammatory airway disorder characterized by obstruction of the airways and is associated with variable and recurring symptoms like bronchial hyper-responsiveness, eosinophil infiltration, mucous hyper-secretion and an underlying inflammation. There is a rapidly increasing prevalence of asthma, especially in the developed countries. WHO estimates that 300 million people currently suffer from asthma worldwide and it is one of the most common chronic disease among children. Striking increases in asthma prevalence has also been observed in populations migrating from a rural to urban environment or from a third-world country to a westernized country. According to the present understanding asthma is caused by a dysregulated Th2 immune response. The “Hygiene Hypothesis” suggests that over past few decades, improved social conditions in the industrialized societies with improved sanitation, use of vaccines and antibiotics has reduced the opportunity for cross infection in young which may be the cause of widespread atopic diseases. However increase in Th1/Th17 mediated disorders like type1 diabetes and inflammatory bowel disorder indicates that there is something more beyond just the Th1-Th2 paradigm, responsible for this augmentation seen in the increasing numbers. Mechanisms that regulate the immune response could be the significant factor affected by the environmental changes in immune tolerance induced by exposure to allergens. Prevention of Th2 biased responses as seen in the airway hyper-responsiveness (AHR) in asthma mediated by CD4<sup>+</sup> T cell tolerance induced by exposure to respiratory allergens, supports this likelihood. Recent data indicate that certain subset of dendritic cells (DC) and regulatory T cells (Treg) can be considered as most important in regulating immune responses in asthma. There is evidence that plasmacytoid dendritic cells (pDC) can induce Treg, however the exact mechanism(s) and cues that favour such a shunt are yet unknown.

**Research focus, specific projects and methods applied:**

Our research focuses on dissecting out the role of regulatory immune cells (pDC and Treg) and mechanisms involved in taming the immune response to alleviate allergic airway inflammation using novel mouse models.

**1) Role of Treg in allergic airway inflammation:**

The canonical markers defining Treg population are CD25 and the transcription factor Foxp3. Since Foxp3 is an intracellular marker, depleting/sorting for Treg using this marker is not possible. Hence most of the research has originally been carried out using anti-CD25 antibodies for depleting these cells. Nevertheless CD25 is also upregulated on activated T cells and hence specific Treg depletion in this paradigm is questionable. We have generated transgenic mice using bacterial artificial chromosome (BAC) technology, which expresses the diphtheria toxin (DT) receptor together with eGFP under Foxp3 promoter (DEREG mouse).

This novel mouse model allows specific detection and inductive depletion of cells expressing Foxp3 (Tregs). Using this mouse we can precisely address the requirement/role of Tregs in restraining asthma pathology. We will induce asthma in DEREg mice using OVA based airway allergy model and deplete FoxP3<sup>+</sup> cells using a standardized regimen of diphtheria toxin treatment. Preliminary data indicates an essential role of Tregs during sensitization phase of allergen induced airway inflammation. Next we want to examine if ablation of Tregs during the challenge phase also result in enhanced asthma pathology. We want to further investigate whether transfer of Tregs can rescue sensitized mice from this pathology, especially during the challenge phase. Since gut infections have been shown to protect from asthma pathology, it will be of interest to study the influence of gut infections (*Salmonella*) on the expansion of Treg.

## **2) Role of pDC and their interplay with Treg in allergic airway inflammation:**

Genetically targeting pDC population was unattainable due to lack of knowledge for pDC specific genetic elements. However, recently our own gene array results and published data from Colonna lab have indicated SiglecH to be exclusively and stably expressed on pDC population. We have generated BAC transgenic mice targeting SiglecH gene locus in combination with Cre-recombinase (pDCre line). We intend to use these mice for specifically depleting/targeting pDC and then to investigate their role in asthma and also scrutinize their role in Treg generation. The novel BAC transgenic founder lines expressing Cre-recombinase under SiglecH promoter have been crossed to RFP-reporter strains (floxed stop-RFP cassette) and the progeny are currently being characterized for transgene expression, thereby giving us the opportunity to track pDC specifically and also to determine the specificity of our pDC-Cre mouse lines. We then aim to cross the pDC-Cre mouse with diphtheria toxin floxed mice, so as to obtain specific depletion of pDC. We intend to use these mice for investigating role of pDCs in asthma pathology. As in DEREg mice, we will deplete pDC in our pDC-Cre X DT mouse and score for asthma symptoms. We will analyze these mice for differences in Treg population and draw its association with the asthma pathology which we will corroborate to the data obtained in DEREg mice.

In both the broad projects we intend to standardize the regimen for inducing asthma in concert with Treg/pDC depletion using various doses of diphtheria toxin. We will confirm the depletion by FACS and score for asthma symptoms by measuring eosinophilia in bronchio-alveolar lavage (BAL), airway hyper-responsiveness, histo-chemistry on lung sections, IgE secretion and various Th1-Th2 cytokines using ELISA and cytometric bead arrays.

### **Time schedule**

1. Year: DT Treatment of DEREg (Balb/c mice) during sensitization/challenge phase
2. Year: Analysis of Treg/pDCre double tg mice (immunohistology, FACS)
3. Year: Depletion of pDCs in pDCre\**iDTR* mice during allergic asthma

### **Group Members:**

*PhD student:* Christian Mayer, Catharina Schrauf, Wiebke Ginter

*MD-Student:* Theresa Förg

*Post-doc:* Abdul Mannan Baru.

*Technicians:* Stephanie Dippel, Martina Thiele.

## Key References for project:

- 1) Lahl K, Loddenkemper C, Drouin C, Freyer J, Arnason J, Eberl G, Hamann A, Wagner H, Huehn J, **Sparwasser T**. Selective depletion of Foxp3<sup>+</sup> regulatory T cells induces a scurfy-like disease. *J Exp Med*. 2007 Jan 22; 204(1):57-63.
- 2) **Sparwasser T**, Gong S, Li JY, Eberl G. General method for the modification of different BAC types and the rapid generation of BAC transgenic mice. *Genesis*; 2004 Jan; 38(1):39-50.
- 3) Hammad H, Lambrecht BN. Dendritic cells and epithelial cells: linking innate and adaptive immunity in asthma. *Nat Rev Immunol*. 2008 Mar;8(3):193-204. Review
- 4) Umetsu DT, McIntire JJ, Akbari O, Macaubas C, DeKruyff RH. Asthma: an epidemic of dysregulated immunity. *Nat Immunol*. 2002 Aug;3(8):715-20. Review.
- 5) de Heer HJ, Hammad H, Soullié T, Hijdra D, Vos N, Willart MA, Hoogsteden HC, Lambrecht BN. Essential role of lung plasmacytoid dendritic cells in preventing asthmatic reactions to harmless inhaled antigen. *JEM*. 2004 July 5;200(1) 89-98
- 6) Blasius AL, Cella M, Maldonado J, Takai T, Colonna M. Siglec-H is an IPC-specific receptor that modulates type I IFN secretion through DAP12. *Blood*. 2006 Mar 15;107(6); 2474-6

## Own references:

- 1) Pellegrini, M., Calzascia, T., Elford, A.R., Shahinian, A., Lin, A.E., Dissanayake, D., Dhanji, S., Nguyen, L.T., Gronski, M.A., Morre, M., Assouline, B., Lahl, K., **Sparwasser, T.**, Ohashi, P.S., and Mak, T.W. Adjuvant IL-7 antagonizes multiple cellular and molecular inhibitory networks to enhance immunotherapies. *Nat. Med.*; Epub 2009 Apr.26.
- 2) Osorio, F., S. LeibundGut-Landmann, M. Lochner, K. Lahl, **T. Sparwasser**, G. Eberl, and C. Reis e Sousa. 2008. DC activated via dectin-1 convert Treg into IL-17 producers. *Eur J Immunol* 38:3274
- 3) Schaefer, M., N. Reiling, C. Kessner, I. Taniuchi, F. Hatam, H. Fehrenbach, J. Ruland, H. Wagner, S. Ehlers, **T. Sparwasser**. Human DC-SIGN transgenic mice are less susceptible to mycobacterial infection. *J Immunol* 2008 15;180(10):6836
- 4) Heit A, F. Gebhardt, K. Lahl, M. Neuenhahn, F. Schmitz, F. Anderl, H. Wagner, **T. Sparwasser\***, D.H. Busch, K. Kastenmüller\*. Circumvention of regulatory CD4<sup>+</sup> T cell activity during cross-priming strongly enhances T cell-mediated immunity. *Eur J Immunol* 2008 38(6):1585.
- 5) Lahl, K., C. Loddenkemper, C. Drouin, J. Freyer, J. Arnason, G. Eberl, A. Hamann, H. Wagner, J. Huehn, **T. Sparwasser**. Selective depletion of Foxp3<sup>+</sup> regulatory T cells induces a scurfy-like disease. *J Exp Med* 2007 204(1):57
- 6) v. Meyenn, F., M. Schaefer, H. Weighardt, S. Bauer, C.J. Kirschning, H. Wagner, **T. Sparwasser**. Toll-like receptor 9 contributes to recognition of Mycobacterium bovis Bacillus Calmette-Guérin by Flt3-ligand generated dendritic cells. *Immunobiology* 2006 211(6-8):557
- 7) Gross, O., A. Gewies, K. Finger, M. Schafer, **T. Sparwasser**, C. Peschel, I. Forster, and J. Ruland. Card9 controls a non-TLR signalling pathway for innate anti-fungal immunity. *Nature* 2006 442:651
- 8) Klemm, S., J. Gutermuth, L. Hultner, **T. Sparwasser**, H. Behrendt, C. Peschel, T.W. Mak, T. Jakob, J. Ruland. The Bcl10-Malt1 complex segregates Fc epsilon RI-mediated nuclear factor kappa B activation and cytokine production from mast cell degranulation. *J Exp Med* 2006 203(2):337
- 9) **Sparwasser, T.**, S. Gong, J.Y.H. Li, G. Eberl. A general method for the modification of different BAC types and the rapid generation of BAC transgenic mice. *Genesis* 2004 38(1):39
- 10) Jung, S., D. Unutmaz, P. Wong, G. Sano, K. De los Santos, **T. Sparwasser**, S. Wu, S. Vuthoori, K. Ko, F. Zavala, E.G. Pamer, D.R. Littman, and R.A. Lang. 2002. In vivo depletion of CD11c(+) dendritic cells abrogates priming of CD8(+) T cells by exogenous cell-associated antigens. *Immunity* 17:211-220.

23.)

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**Engagement in the MD/PhD program or HBRs:**

Prof. Dr. Ingo Just

Supervisor: Yes Name of student(s): Gesa Meyer,  
Lecturer: X; PhD Kommission: ; No, not yet:

PD Dr. Andreas Pich

Supervisor: Yes Name of student(s): Frank Traub,  
Lecturer: no; PhD Kommission: ; no, not yet:

**Animal experiments involved:**  yes  no

**Research focus:**

Our research focuses on the mode of action of bacterial protein toxins. Those toxins harbour enzymatic activity, which is executed after self-mediated cell-entry into eukaryotic target cells. The toxins studied enzymatically transfer sugars onto the small GTP-binding proteins of the Rho subfamily. Rho proteins are nucleotide-driven molecular switches, which are the master regulators of the actin cytoskeleton and which are also involved in the regulation of gene expression, cell cycle progression and cell death. Toxin-catalyzed Rho modifications comprise mono-glucosylation (toxin A from *Clostridium difficile*) and ADP-ribosylation (C3 exoenzyme from *Clostridium botulinum*, which is not related to neurotoxins). C3 enzyme harbours an exceptional position because it is the only intracellular enzymatically acting toxin, which does not possess a transport domain. Such a domain is present in all other comparable toxins, which is in charge of binding to membrane receptors and the subsequent receptor-mediated endocytosis. Further exceptional is the extremely restricted substrate specificity. C3 exclusively ADP-ribosylates RhoA,B and C (>90% homologous to each other) but not the 20 members of the Rho subfamily nor the about 160 members of the Ras superfamily of small GTP-binding proteins. Our group has elucidated the recognition of Rho by the C3 exoenzyme and the molecular consequences of Rho ADP-ribosylation. The substrate specificity has been the basis for the usage of C3 as pharmacological tool in cell biology; the poor cell accessibility has been overcome by creating cell permeable fusion toxins. The extreme high target Rho specificity is also the reason for the development of (cell permeable) C3 as drug for the treatment of spinal cord injury.

**Specific project and methods applied:**

**Title:** Influence of C3 on the protein profile of neuronal cells

**Aims:** .....

**Funding:** DFG

We have recently detected that the well-known neurotrophic effect of C3 on neuronal cells is not based on its inherent enzyme activity as the enzyme-deficient C3 is also able to exhibit neurotrophic activity. Neurotrophic activity means that C3 is able to enhance growth of axons and dendrites as well as enhances axonic and dendritic branching. Such activity of C3 is only detectable for hippocampal and motoneurons. We dissected the C3 molecule and identified a peptide encompassing 26 amino acids, which harbours the identical neurotrophic activity of full-length C3 with respect to concentration and potency. The activity is not only detected in cultured neurons but also in animal models: in a peripheral neuronal lesion model (rat) as well as in the spinal cord injury model (mouse) the peptide from C3 is superior to standard treatments. After the proof of principle we now focus on the cellular level of the action of C3 peptide. Especially we want to identify the C3 receptor (which is

still unknown) and those signal pathways triggered by C3 peptide. To this end, changes in the protein profile of neuronal cells induced by C3 peptide are to be monitored by a mass-spec-based proteomic approach.

The ICPL- and SILAC-technique will be used to compare protein profiles of C3- treated and untreated cells. These methods allow quantitative proteomics based on stable heavy and light isotopes and will be used to elucidate the effects of C3. Additionally, the SILAC-technique enables pulse chase labeling experiments to identify transient cellular processes depending on C3 treatment.

Time- and concentration-dependent dissolution of the changes in the protein profile gives an impression about the overall as well as information on cell-compartment based alterations.

Furthermore, the project on the identification of the C3-receptor (continuation of a running project) should be continued by mass-spec-based identification of the C3 membranous binding protein.

### **Time schedule**

1. C3 Exoenzyme receptor Isolation and identification  
12 months
2. Comparative Proteome analysis of C3-treated vs. untreated neuronal cells  
24 months

### **Group Members:**

Nelli Jochim, PhD student; Johannes Zeiser, PhD student

Anke Schröder, Diploma student; Angela Fülbier, Diploma student

Karin Agternkamp, technician

### **Key References for project**

McKerracher L (2001) Spinal cord repair: strategies to promote axon regeneration. *Neurobiol Dis* 8: 11-18

McKerracher L and Horiguchi H (2006) Targeting Rho to stimulate repair after spinal cord injury. *J Neurotrauma* 23:309-317

Ruff RL, L. McKerracher, and ME selzer (2008) Repair and neurorehabilitation strategies for spinal cord injury. *Ann. N.Y. Acad. Sci* 1142:1-20

Ahnert-Hilger, G., Höltje, M., Große, G., Pickert, G., Mucke, C., Nixdorf-Bergweiler, B., Boquet, P., Hofmann, F., and Just, I. (2004) Differential effects of Rho GTPases on axonal and dendritic development in hippocampal neurons. *J. Neurochem.* 90:9-18

Höltje M, Djalali S, Hofmann F, Münster-Wandowski A, Hendrix S, Boato F, Dreger S, Große G, Henneberger C, Grantyn R, Just I, Ahnert-Hilger G (2009) A 29 amino acid fragment of Clostridium botulinum C3 protein enhances neuronal outgrowth, connectivity and reinnervation. *FASEB J* in press

### **Own references (mainly 2004-2009):**

Höltje M, Djalali S, Hofmann F, Münster-Wandowski A, Hendrix S, Boato F, Dreger S, Große G, Henneberger C, Grantyn R, Just I, Ahnert-Hilger G (2009) A 29 amino acid fragment of Clostridium botulinum C3 protein enhances neuronal outgrowth, connectivity and reinnervation. *FASEB J* in press

Höltje M, Hofmann F, Lux R, Veh RW, Just I, Ahnert-Hilger, G (2008) Glutamate uptake and release by astrocytes are enhanced by Clostridium botulinum C3 protein. *J Biol Chem.* 283:9289-99.

Hoffmann A, Hofmann F, Just I, Lehnardt S, Hanisch UK, Brück W, Kettenmann H, Ahnert-Hilger, G. Höltje M (2008) Inhibition of Rho-dependent pathways by Clostridium botulinum C3 protein induces a proinflammatory profile in microglia. *Glia* 56:1162-75.

Aktories, K.; and I. Just. (2005). Clostridial Rho-inhibiting protein toxins. *Curr. Top. Microbiol. Immunol.* 291: 113-145.

Höltje, M., Hoffmann, A., Hofmann, F., Mucke, C., Grosse, G., Van Rooijen, N., Kettenmann, H., Just, I., Ahnert-Hilger G. (2005) Role of Rho GTPases in astrocyte morphology and migratory response during in vitro wound healing. *J. Neurochem.* 95:1237-48.

Ahnert-Hilger, G., Höltje, M., Große, G., Pickert, G., Mucke, C., Nixdorf-Bergweiler, B., Boquet, P., Hofmann, F., and Just, I. (2004) Differential effects of Rho GTPases on axonal and dendritic development in hippocampal neurons. *J. Neurochem.* 90:9-18

Muetzelburg MV, Hofmann F, Hahner S, Just I, Pich A (2009) Quantitative proteome analysis of human neuroblastoma cells treated with Clostridium botulinum C3 exoenzyme – application of isotope-coded protein labels and LC-MALDI techniques, Manuscript submitted for publication in *Proteomics*

Witzendorff D von, Maass K, Pich A, Ebeling S, Kölle S, Kochel C, Ekhlesi-Hundrieser M, Geyer H, Geyer R, Edda Töpfer-Petersen (2009) Characterization of the acidic N-linked glycans of the zona pellucida of prepuberal pigs by a mass spectrometrical approach. *Carbohydrate Research* accepted

Muetzelburg MV, Hofmann F, Just I, Pich A (2009) Isobaric tags for relative and absolute quantitation coupled with 2D-LC-MALDI-MS/MS techniques enable the identification of biomarkers of C3 exoenzyme treated human neuroblastoma cell lines., *J Chromtogr B.* 877, 1344-1351

Neumann D, Kollwe C, Pich A, Cao P, Resch K, and Martin MU (2008) Threonine 66 in the death domain of IRAK-1 is critical for Interaction with other Signaling Molecules but is not a Target Site for phosphorylation, *J. Leukocyte Biol.*, 84, 807-813

Klisch K, Jeanrond E, Pang PC, Pich A, Schuler G, Dantzer V, Kowalewski MP, Dell A (2008) A tetraantennary glycan with bisecting N-Acetylglucosamine and the Sd<sup>a</sup> antigen is the predominant N-glycan on bovine Pregnancy-associated glycoproteins, *Glycobiology* 18, 42-52

Stolz A, Haines N, Pich A, Irvine KD, Hokke, CH, Deelder AM, Gerardy-Schahn R, Wuhler M, Bakker H (2008) Distinct contributions of b4GalNAcTA and b4GalNAcTB to Drosophila glycosphingolipid biosynthesis. *Glycoconj J* 25, 167-75

Jin Rongsheng, Sikorra S, Stegmann CM, Pich A, Binz T, Brunger AT (2007) Neurotoxin serotype C1 light chain protease: implications for dual substrate specificity. *Biochemistry* 46, 10685-10693

Frommberger M, Zürgbig P, Jantos J, Krahn T, Mischak H, Pich A, Just I, Schmitt-Kopplin P and Schiffer E (2007) Peptidomic analysis of rat urine using capillary electrophoresis coupled to mass spectrometry. *Proteomics Clinical Application*, 1, 650-660

24.)

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**Engagement in the MD/PhD program or HBRS:**

Supervisor: X Name of student(s): Aliaksandra Maroz, Benjamin Groß (Regenerative Science)

Lecturer: ; PhD Kommission: ; No, not yet:

**Animal experiments involved:** X yes  no

**Research focus:**

Our research focuses on the genetic control mechanisms underlying the development of acute myeloid leukemia (AML) and the normal hematopoiesis. The AML-BFM study group is worldwide the largest group of its kind holding the most comprehensive patient sample collection.

Children with Down syndrome (DS) are at high risk to develop myeloid leukemia (ML-DS) and a postnatal transient leukemia (TL). TL and ML-DS are therefore an ideal model to study leukemogenesis. The molecular mechanisms predisposing to leukemia in these children are largely unknown. Recently, acquired mutations in the hematopoietic transcription factor *GATA1* –leading to the exclusive expression of a shorter isoform (GATA1s)- have been found in nearly all children with ML-DS and TL. However, both *Gata1s* knock-in mice and *Gata1s* mice crossed with currently available DS mouse models (Ts65dn) develop leukemia.

The search for new genes involved in the development of ML-DS led us to look for microRNAs encoded on chromosome 21. As cancer is essentially a consequence of disordered genome function, one might expect these regulatory molecules to be involved in the development of this disease. Our studies showed that *miR-125b-2* - located on human chromosome 21 - is overexpressed in ML-DS and ectopic expression leads to hyperproliferation and altered differentiation program of human hematopoietic stem and progenitor cells (HSPC). Using an integrated genomic approach we discovered a set of target genes, of which *DICER1*, *ST18* and *LIFR* were experimentally validated. Those genes were found to be downregulated in authentic ML-DS. Thus, we discovered chromosome 21-encoded *miR-125b-2* as an oncoMiR involved in trisomy 21-associated AML.

**Specific project and methods applied:**

**Title:** miRNAs and their role in normal and malignant hematopoiesis

**Aims:** In the proposed project the function and effects of all known miRNAs in normal and malignant hematopoiesis should be investigated.

**Funding:** DFG

We have recently shown the positive effect of *miR-125b* on the proliferation of both HSPC and leukemic cell lines. Further we screened for new miRNAs using a miRNA expression profiling [TLDA] and identified novel candidates that may be involved in the pathogenesis of myeloid leukemia. The aims of the proposed project are:

**1. Analysis of function and relevance of identified miRNAs in normal and malignant hematopoiesis by knock-down or overexpression experiments.**

The pri-miRNA with the flanking DNA sequence will be cloned into a retroviral vector (MSCV) and ectopically expressed in CD34<sup>+</sup>-HSPC. Transduced cells will be further analyzed using colony forming assays (hematopoietic and megakaryocytic), *in vitro* differentiation, immunophenotyping, cell cycle analyses, cell viability assays. For knock-down experiments antiMiRs will be transfected into appropriate cell lines (myeloid leukemia according to the subtype M1-M7), which are further examined by immunophenotyping, cell cycle analyses, cell viability assays.

2. Identification and of target genes, which are essential for the effect of chromosome 21 encoded *miR-99a*, *miR-155* and *let-7c* and of those newly identified miRNAs (see 1.)

Target genes will be identified using publically available databases and prediction tools in combination with gene-expression analyses of the transduced HSPC and the transfected cell lines (see 1.). Target genes will be validated using luciferase reporter assays with the 3'UTR including site directed mutagenesis of the predicted miRNA binding site. In case a target gene has been identified, it will also be included in our functional studies using RNAi (shRNA) and retroviral overexpression (rescue studies).

Methods that will be required for the successful conduction of the project include: retroviral transduction of HSPC and cell lines (cloning and sequencing of the vectors, cell sorting of GFP<sup>+</sup>-HSPC), transfection (elektroporation) of *antimiRs* (selection Cy5<sup>+</sup>-HSC by cell sorting), gene expression analysis by microarray technology with data analysis, immunophenotyping (FACS), colony assays, site directed mutagenesis of the miRNA seed region, luciferase reporter assays (3'UTR of potential target genes), qRT-PCR, cell cycle analyses, co-immunoprecipitation (CoIP), chromatin-immunoprecipitation (ChIP), Western-Blot.

### Group Members:

*Katarina Reinhardt, PhD, Post doctorant, Stefanie Ehlers, MD, Post doctorant*

*Aliaksandra Maroz, PhD student; Benjamin Groß, PhD student, Raghavan T.V., PhD student  
Kirsten Heitmann, MD student*

*Sabine Knönagel, technician; Caroline Augsburg, technician; Martina Wackerhahn, technician; Tanja Reinker, technician*

### Key References for project

1. Croce CM. Oncogenes and cancer. **N Engl J Med.** 2008;358:502-511.
2. Carthew RW, Sontheimer EJ. Origins and Mechanisms of miRNAs and siRNAs. **Cell.** 2009;136:642-655.

### Own references:

3. Li Z, Godinho FJ, **Klusmann JH** et al. Developmental stage-selective effect of somatically mutated leukemogenic transcription factor GATA1. **Nat Genet.** 2005;37:613-619.
4. **Klusmann JH**, Creutzig U, Zimmermann M, Dworzak M, Jorch N, Langebrake C, Pekrun A, Macakova-Reinhardt K, **Reinhardt D**. Treatment and prognostic impact of transient leukemia in neonates with Down syndrome. **Blood.** 2008;111:2991-2998.
5. **Klusmann JH**, Reinhardt D, Hasle H et al. Janus kinase mutations in the development of acute megakaryoblastic leukemia in children with and without Down's syndrome. **Leukemia.** 2007;21:1584-1587.
6. **Klusmann JH**, Langebrake CWK, Kolar M, Puhmann U, Reinhardt D. Concomitant aberrant overexpression of RUNX1 and NCAM in regenerating bone marrow of myeloid leukemia of Down's syndrome. **Haematologica.** 2006;91:1473-1480.
7. **Klusmann JH**, Heitmann K, Godinho FJ, Pushpanathan T, **Reinhardt D**, Orkin SH. Developmental stage-specific interplay between GATA1 and IGF signaling in fetal hematopoiesis and leukemogenesis. **Cancer Cell.** 2009; **submitted.**

**8. Klusmann JH**, Janikova K, Li Z, Orkin SH, Reinhardt D. Chromosome 21-Encoded miR-125b and Its Role in the Development of Myeloid Leukemia in Children with Down's Syndrome. ASH Annual Meeting Abstracts. 2007;110:716. **Best of ASH**

## Timeline:

	1. Jahr		2. Jahr		3. Jahr	
	1. Halbjahr	2. Halbjahr	1. Halbjahr	2. Halbjahr	1. Halbjahr	2. Halbjahr
<b>Screening nach weiteren für die Pathogenese der Leukämie entscheidenden miRNAs</b>	miRNA-Expressionprofil bioinformatische Analysen und Identifizierung von miRNA für weitere Versuche	Klonierung von miRNAs, Herstellung von hochtitrigem retroviralem Überstand	Retrovirale Transduktion humaner CD34 <sup>+</sup> -HSC, megakaryozytäre und hämatopoetische Colony-Assays, replating-efficiency			
			Kultur der Zelllinien (CMK, UT-7, M-07e, K562), Transfektion mit 2-O-Methyl Oligonukleotiden, Durchflusszytometrie (Apoptose, Differenzierung), Morphologie			
<b>Die Analyse der Funktion der miRNAs bei der Hämatopoese und Megakaryopoese</b>			Transplantationsversuche mit retroviral transduzierten HSPC; Analyse: Leukämieentstehung, Blutbilder, Knochenmark <i>in vitro</i> : megakaryozytäre und hämatopoetische Colony-Assays, replating-efficiency			
<b>Identifizierung von Ziel- Genen und Proteinen , die entscheidend für den Effekt bereits identifizierter miRNAs sind</b>	Kultur der Zelllinien (CMK, UT-7, M-07e, K562) und murinen FL-HSC; Transfektion mit 2-O-Methyl Oligonukleotiden , RNA- und Proteinisolierung	Microarray, bioinformatische Analyse der Daten. Generierung der Liste der miRNA-Zielgene LC-MALDI Analyse der Proteine mit unterschiedlichen Funktionalitäten	Klonierung von Genen und shRNA; Herstellung von hochtitrigem retroviralem Überstand	Retrovirale Transduktion humaner CD34 <sup>+</sup> -HSPC, megakaryozytäre und hämatopoetische Colony-Assays, replating-efficiency		
	Retrovirale Transduktion humaner CD34 <sup>+</sup> -HSC und muriner FL-HSC mit miR-99a, miR-125b-2, miR-155 und let-7c			Transplantationsversuche mit retroviral transduzierten HSPC; Analyse: Leukämieentstehung, Blutbilder, Knochenmark <i>in vitro</i> : megakaryozytäre und hämatopoetische Colony-Assays, replating-efficiency		