Molecular Haematopoiesis and Epigenetics

Group Leader: **Professor Constanze Bonifer, Chair of Experimental Haematology (/staff/profiles/cancer/bonifer-constanze.aspx)**

Overview

Our main research interest is to study the mechanism of cell fate decisions at the level of gene regulation. All blood cells arise from pluripotent stem cells of the bone marrow. We want to understand in mechanistic detail how different genetic programs are activated and silenced at specific stages of blood cell development and which factors are involved in this process. In addition, we study how this finely balanced process is subverted in leukaemic cells.

Our research group

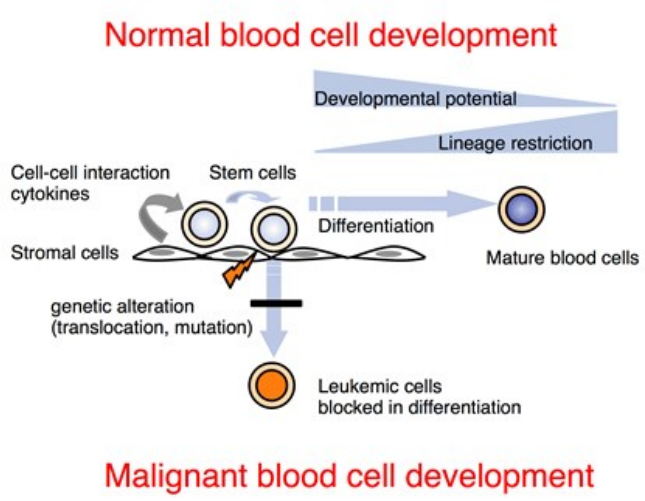
In our work, we address the question of how the regulators of transcription, the sequence-specific DNA binding proteins or transcription factors, interact with the chromatin template and change its structure. We know from genetic studies that chromatin modification complexes play essential roles in all phases of the development of multicellular organisms.

We also know that transcription factors bring these epigenetic regulatory proteins to specific genes. Together, they are responsible for the expression of different genes. Our research has shown that even the process of expressing one gene at the right time and in the right cell is a breathtakingly complex process that involves the coordinate action of hundreds of different molecules. We have also made progress in understanding how these intricately balanced processes are disturbed in leukaemia.

We have now taken these studies one step further. One of the great challenges for future biological and medical research will be to understand how all genes and all molecules in a cell work together to generate different cells that each express only one set of genes. This means that we will have to study all genes simultaneously. To this end, we employ sophisticated genome-wide methods such as ChIP-sequencing and DNaseI-sequencing to generate such data.

We also collaborate with computational biologists to reconstruct models of the molecular interactions driving blood cell development. However, we also study the global consequences of expression of aberrant transcription factors in form of nuclear oncogenes on how the epigenetic landscape is altered in leukaemic cells. The outcome of such studies will shed light on the complex deregulation processes that turn normal into leukaemic cells and will uncover novel therapeutic targets to combat a disease with a high death toll, in particular amongst the elderly.

The results of our experiments are therefore not only important for our understanding of how blood cells form, but are extremely important for how we may diagnose and treat patients in the future.



Current projects

Open all sections

1. Establishment of the Haematopoietic Transcriptional Programme: From Systems Approaches to Molecular Mechanisms (together with B.Göttgens, Cambridge; G.Lacaud and V.Kouskoff, Manchester; D.Westhead, Leeds). BBSRC LoLa grant.

Postdocs: Monika Lichtinger, Nadine Obier. Technician: Laura Noailles.

This project is funded by a BBSRC strategic LoLa and examines the ordered interplay of transcription factors and specific chromatin states leads to the stable expression of lineage specific genetic programs. We use haemopoiesis as a model to identify the molecular mechanisms and dynamics of cell differentiation in a system-wide fashion. To this end we have formed a consortium consisting of experimental researchers and computational biologists to study haemopoietic development as particularly powerful system for the reconstruction of dynamic and global models of the molecular interactions governing an entire developmental pathway.

2. Mechanistic insights into the interplay between transcription factors and the epigenetic regulatory machinery in normal and leukaemic cells (together with Peter Cockerill). Leukemia Lymphoma Research Specialist Programme.

Postdocs: Anetta Ptasinska, Pierre Cauchy, So Yeon Kwon, Joaquin Zacarias, Maria Rosaria Imparato. PhD students: Anna Pickin and Niall Gildin. Collaborators: Olaf Heidenreich, University of Newcastle, Dan Tenen, Harvard Stem Cell Institute and National University of Singapore, Manoj Raghavan, Haematology, Birmingham; David Westhead, University of Leeds.

In this program, we examine the molecular mechanisms underlying how normal and aberrant leukaemic transcription factors interact with the epigenetic regulatory machinery, reprogram the epigenetic landscape of normal human precursor cells and initiate the vast deregulation phenomena that we observe in malignant cells. We are also developing methods and computational tools that will allow us to model how the human epigenome swings back to normal, once we eliminate specific leukaemia-initiating oncogenes and/or block aberrant signalling through therapy.

3. The role of the transcription factor Sp1 in embryonic haematopoiesis. MRC.

Postdocs: Jane Gilmour, Salam Assi, in collaboration with Sjaak Philipsen, Erasmus University Rotterdam, and David Westhead, University of Leeds.

Mammalian development is regulated by the interplay of tissue-specific and ubiquitously expressed transcription factors, such as Sp1. Sp1 knock-out mice die in utero with multiple phenotypic aberrations, but the underlying molecular mechanism of this differentiation failure has been elusive. In this study we use the differentiation of mouse ES cells as a model to address this issue. To this end we examined differentiation potential, global gene expression patterns and Sp1 target regions in Sp1 wild-type and deficient cells representing different stages of hematopoiesis. Sp1-/- cells progress through most embryonic stages of blood cell development but cannot complete terminal differentiation. For most Sp1 target and non-target genes, gene expression is unaffected by Sp1 inactivation. However, Cdx and multiple Hox genes are stage-specific targets of Sp1 and are down-regulated at an early stage. As a consequence, expression of genes involved in hematopoietic specification are progressively deregulated, highlighting the regulatory hierarchy of hematopoietic specification. However, our data show that also a large number of genes encoding metabolic pathways are deregulated. Our work demonstrates that the early absence of active Sp1 sets a cascade in motion that culminates in a failure of terminal hematopoietic differentiation and emphasizes the role of ubiquitously expressed transcription factors for tissue-specific gene regulation.

4. Mechanistic insights into the deregulation of haematopoietic development by mutated forms of the RUNX1 transcription factor. Kay Kendall Leukaemia Fund.

Postdoc: Regha Kakkad, in collaboration with Georges Lacaud, Paterson Institute of Cancer Research, Manchester

The transcription factor RUNX1 is crucial for the establishment of haemopoiesis and mutation of this gene plays an important role in myeloid leukemia. However, little is known about the mechanistic details of how mutant versions of RUNX1 subvert normal haemopoietic development and counteract normal RUNX1 activity. Using the differentiation of mouse embryonic stem (ES) cells as model we address this question.

5. Geneome-wide maps of transcription factor binding in the human epigenome.

PhD student: Jason Piper, in collaboration with Sascha Ott, Systems Biology Institute, University of Warwick.

In this project we are employing nuclease digestion followed by high-throughput sequencing at high read depth to probe the chromatin fine structure of normal and malignant cells. This tells us how the epigenetic landscape differs between normal and cancer cells, and which transcription factor biding sites are occupied and which ones are not. Some of this work has been published in Piper et al., (2013) Nucleic Acids Res. 41, e201.

6. The role of aberrant transcription factor expression and loss of epigenetic control in activating long-terminal-repeats in classical Hodgkin lymphoma

PhD student Ben Egington-White, in collaboration with Mike Griffiths, West Midlands Regional Genetics Service, Arthur Riggs, Beckman Institute of City of Hope, Stephan Mathas, Charite Berlin

Hodgkin lymphoma is characterized by the presence of malignant Hodgkin-/Reed-Sternberg (HRS) cells The majority of the tumour tissue is composed of normal immune cells, which are attracted by HRS cell secreting cytokines and chemokines. Our previous work demonstrated that in classical Hodgkin lymphoma extensive chronic signaling processes and aberrant transcription factor activity combined with loss of epigenetic regulation leads to a reprogramming of the epigenome and lineage inappropriate gene expression. This also included a wide-spread activation of endogenous long-terminal repeats (LTRs) which drive the expression of at least one gene important for tumour pathology. Here we address the question how aberrantly expressed transcription factors and activated LTRs reshape the epigenetic and genetic landscape in this type of cancer. The main objective of our work is to examine a genome-wide link between aberrant transcription factor activity, altered epigenetic control, LTR activation and genome stability. We believe that they comprise important proof-of-principle studies which may pave the way for developing new diagnostic tools for the analysis of LTR activation as a new risk factor for malignant cells.

7. Identification of common and distinct epigenetic reprogramming properties of core-binding-factor fusion proteins

Clinical Research fellow: Justin Loke. Collaborators: Ruud Delwel, Erasmus University Rotterdam.

Mutations involving RUNX1 are the most frequent case of acute myeloid leukaemia (AML) but how these molecules block differentiation is only poorly understood. RUNX1 activity can be altered as a result of translocations. This results in targeting ectopic activities to RUNX1 binding sites by fusing its DNA-binding (RUNT) domain to those of other protein domains. Currently, it is not known whether different RUNX1 translocation products bind to similar targets and deregulate similar pathways. We have recently shown that expression of the translocation product RUNX1/ETO leads to the reprogramming of the epigenetic landscape and to alterations of transcription factor binding at thousands of genomic sites. Knock-down of RUNX1/ETO largely reverses reprogramming. In this proposal, we will study a second RUNX1 translocation, the t(3;21) which fuses the RUNT domain to the entire EVI-1 gene resulting in the expression of the fusion protein RUNX1/EVI-1. Using genome-wide assays, we will determine the RUNX1/EVI-1 specific cistrome and compare it to that of RUNX1/ETO. We also designed a functional siRNA specifically targeting RUNX1/EVI-1 to deplete it and test the effect on gene expression and chromatin programming. The aim of this work is to identify common and targetable pathways affected by two different translocation products.

PhD projects are on offer in all of the research areas described above.

Recent publications

- **Zhang H, Alberich-Jorda M, Amabile G, Yang H, Staber PB, Diruscio A, Welner RS, Ebralidze A, Zhang J, Levantini E, Lefebvre V, Valk PJ, Delwel R, Hoogenkamp M, Nerlov C, Cammenga J, Saez B, Scadden DT, Bonifer C, Ye M, Tenen DG**. Sox4 is a key oncogenic target in C/EBPα mutant acute myeloid leukemia. Cancer Cell. 2013; 24(5):575-88.
- **Ray D, Kwon SY, Tagoh H, Heidenreich O, Ptasinska A, Bonifer C**. Lineage-inappropriate PAX5 expression in t(8;21) acute myeloid leukemia requires signaling-mediated abrogation of polycomb repression. Blood. 2013; 122(5):759-69.
- **Lichtinger, M., Ingram, R.M., Hannah, R., Clarke, D., Müller, D., Lie-A-Ling, M., Noailles, L., Zhang, P., Wu, M., Tenen, D.G., Assi, S., Westhead, D.R., Kouskoff, V., Lacaud, G., Göttgens, B., and Bonifer, C.** (2012) RUNX1 reshapes the epigenetic landscape at the onset of haematopoietic development. EMBO J. In press.
- **Ptasinska, A.; Assi, S.A., James, S.R.,Williamson, D.,Hoogenkamp, M., Mengchu, W., Care, M., McNeill, H., Cullen, M., Tooze, R., Tenen, D.G., Cockerill, P.N. Westhead, D.R.,Heidenreich, O. and Bonifer, C.** (2012). Reversible genome-wide epigenetic reprogramming by the leukemia-initiating fusion protein RUNX1/ETO. Leukemia 26:1829-41
- **Leddin, M., Perrod, C. , Hoogenkamp, M., Ghani, S., Ass, S., Heinz,S., Wilson, N.K., Follows, G., Schönheit,J., Vockentanz,L., Mosamam,A., Chen, W., Tenen, D.G., Westhead, D.R., Göttgens, B., Bonifer, C*. and Rosenbauer, F*.** (2011) (*joint corr. authors). Two distinct auto-regulatory loops operate at the Pu.1 locus in B cells and myeloid cells. Blood. Mar 10;117(10):2827-38
- **Lamprecht, B., Walter, K., Kreher, S., Kumar, R., Hummel, M., Lenze, D., Köchert, K., Bouhlel, M.A., Richter, J., Soler, E., Stadhouders, R., Jöhrens, C., Wurster, K.D., Callen, C., Harte, M.F., Giefing, M., Barlow, R., Stein, H., Anagnostopoulos, I., Janz, M., Cockerill, P.N., Siebert, R., Dörken, B., Bonifer, C.*, and Mathas, S.*** (2010). (*Joint corresponding authors). De–repression of an endogenous long terminal repeat activates the CSF1R proto–oncogene in human lymphoma. Nature Medicine. 16, 571 – 579

Staff

Other Research Group Staff Members

Postdocs: Monika Lichtinger, Nadine Obier, Anetta Ptasinska, Pierre Cauchy, So Yeon Kwon, Joaquin Zacarias, Maria Rosario Imparato, Regha Kakkad, Jane Gilmour, Salam Assi

PhD Students: Anna Pickin, Ben Edington-White, Jason Piper (with Warwick)

Clinical fellow: Justin Loke

Technical staff: Laura Noailles