

Molecular Genetics of Hematopoiesis & Leukemogenesis

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Introduction

A number of transcription factors preferentially expressed in the hematopoietic system are pivotal in hematopoietic stem cell biology, cell differentiation, and the emergence of leukemia. These transcription factors participate in networks of gene regulatory complexes which direct cell fate decisions in growth control and in the differentiation of blood cells. These hematopoietic transcription factors include Myb, C/EBP α , C/EBP β , Runx2 (AML1), Scl/Tal1, PU.1, and GATA1 and many of them are found as mutated versions in leukemia.

Deregulated differentiation of blood cells or defective control of their self-renewal and proliferation are hallmarks of leukemia. Experimental and genetic evidence suggests that mutations in hematopoietic transcription factors are major causes of their dys-regulation. Accordingly, a prerequisite for the development of rational cures for leukemic diseases is a deeper understanding of the molecular genetics of these critical transcription factors and of the mechanisms how normal or mutated transcription factors regulate the hematopoietic cell biology. We are aiming to answer the following questions: How do hematopoietic cells arise? What are the genetic and epigenetic programs that these transcription factors regulate? How are proliferation and differentiation connected, how are they regulated and how are they dys-regulated during leukemogenesis? How is cell identity achieved? What are the signals and mechanisms of stem cell maintenance, commitment and differentiation? How can we modify these processes?

Generation of Blood Cells.

Blood cells emerge early during embryogenesis in the yolk sac (in the mouse approximately at day 7.5 of a total gestation time of 20 days). These early stage or embryonic hematopoietic cells are replaced by the adult hematopoietic system that lasts a lifetime. Adult hematopoietic stem cells first arise in the aorta-gonad-mesonephros (AGM) region (approximately at day 10.5 in the mouse) from so-called hemangioblast progenitors. Hemangioblasts generate both endothelial cells and hematopoietic stem cells. The latter then migrate to the liver and subsequently to the bone marrow where they generate all types of blood cells and also

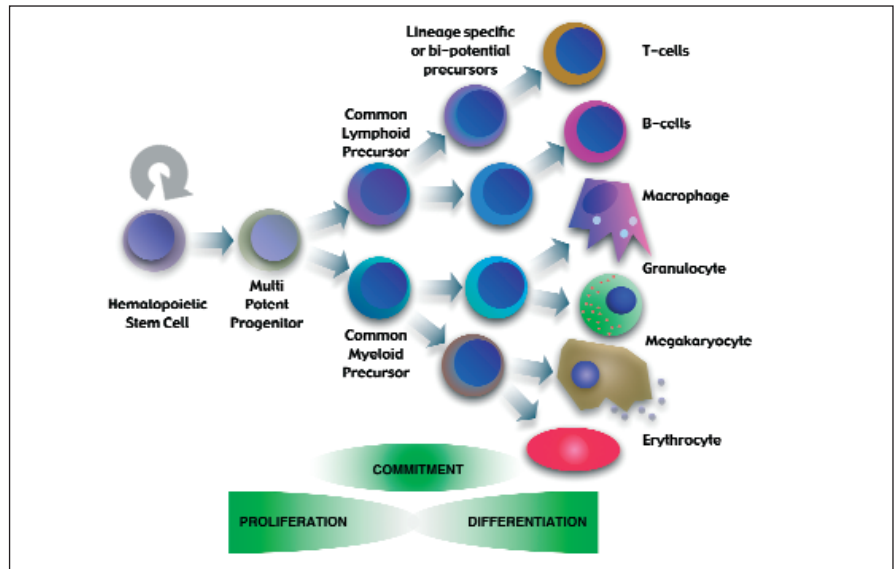


Fig. 1: A hierarchical model of hematopoiesis.

Hematopoietic stem cells that reside in the bone marrow may self renew (circular arrow) and/or give rise to restricted progeny. Multipotential progenitors may generate cells of all lineages but have lost their self renewal capacity. Common lymphoid- and myeloid precursor cells are further restricted for differentiation into various B- and T-cells or into myeloid cells, respectively. Finally, lineage restricted precursors give rise to mature blood cell types as indicated on the right. Note that precursor and progenitor compartments expand exponentially, whereas end cells are largely proliferation arrested. Experimental evidence suggests that during the commitment phase cells undergo binary decisions that are executed by distinct hematopoietic transcription factors.

uphold their own maintenance in a condition called self-renewal.

Hematopoietic stem cells give rise to early multi-lineage progenitor cells and to more restricted precursor cells (Figure 1). The initial stem cell progeny has a high capacity for proliferation in addition to a multi-lineage differentiation potential. Progenitor- and precursor cells, however, undergo a number of binary lineage decisions and become increasingly restricted for differentiation into distinct blood cell types such as, e. g. erythrocytes, neutrophils, or macrophages. When an individual develops Leukemia these hierarchical processes of supervised development run out of control. Leukemia cells no longer comply with the hematopoietic hierarchy and display runaway proliferation of early cells. Thus, hemtopoiesis provides an excellent model system to address fundamental cell biological questions that relate to stem cell biology, cell lineage commitment, and to cell differentiation control. Studies of hematopoietic regeneration and experimental bone marrow transfer have revealed many of the biological principles of stem cell biology. Answers to these questions

are also clinically relevant e.g. for the understanding of the development of leukemia and for stem cell therapy.

Combinatorial control of gene expression

Cell development and differentiation programs are accomplished by switching on and off distinct sets of genes. Gene regulatory proteins (transcription factors) that are downstream of signaling cascades bind to control regions of developmentally important genes and suppress or activate their expression. Transcription factors may also leave epigenetic marks at gene loci that determine how the gene they interact with can be regulated in the future.

Specificity of gene expression is primarily achieved by combinatorial control, i.e., through physical and functional interactions between several transcription factors that are simultaneously or sequentially bound to the same gene loci. Combinatorial gene switches permit plasticity of regulation and allow a multitude of developmental decisions to be taken with a limited number of regulators.

Many of such developmentally important gene regulatory proteins are also prone to tumorigenic conversion by mutations. Mutations in critical transcription factors can disrupt their normal function in gene expression control and epigenesis and can cause uncontrolled cell multiplication. Developmentally essential hematopoietic gene regulatory proteins are therefore often involved in leukemogenesis. The first leukemia genes were discovered some 60 years ago in acutely transforming oncogenic retrovirus strains that transduce high-jacked cellular genes encoding hematopoietic transcription factors. These retroviral oncogenes arose by recombinatorial events between the retroviral genome and transcription factor genes, suggesting that cancer is a disease of genetic alterations in somatic cells. Research on retroviral and cellular oncogenes in conjunction with the molecular biological techniques developed during the last 25 years have helped to uncover important mechanisms of oncoproteins. This research has introduced and forwarded the oncogene hypothesis as the basis of rational cancer research. It states that during tumorigenesis, somatic cells pick up multiple mutations that disrupt signal pathways, induce faulty gene regulation, overturn epigenetics, or deregulate proliferation and differentiation. Many genes involved in tumorigenic conversion have since been discovered and most of them can be linked to the dysregulation of the following five basic cellular functions: control of proliferation, differentiation, apoptosis, senescence, and maintenance of genome integrity. Many of the recurrent leukemia genes are mutated versions of transcription factors normally regulating hematopoiesis. It is therefore

essential to understand the mechanism of how such transcription factors regulate the expression of genes. Several years ago, we were able to identify the first two transcription factors responsible for a combinatorial molecular switch instructing cells to express myeloid genes. Other transcription factors followed and led to the concept of combinatorial control of hematopoietic lineage acquisition and cell maturation. The myeloid gene switch consists of two types of transcription factors that are also involved in leukemogenesis, namely: 1) proteins of the CCAAT-/Enhancer Binding Protein family (C/EBP) that regulate differentiation and cell cycle arrest and 2) the product of the Myb proto-oncogene that is essential for the development and maintenance of all hematopoietic lineages. In a concerted action, both transcription factors may instruct even fibroblasts to express myeloid genes. We have exploited this potential of ectopically inducing hematopoietic gene expression in non-hematopoietic cells to explore and dissect how both Myb and C/EBP collaborate during gene regulation. The cellular c-Myb gene encodes an essential hematopoietic transcription factor involved in self renewal of stem cells that also collaborates with C/EBP to regulate differentiation. Mutations in either transcription factor may abrogate their collaboration, disrupt the myeloid differentiation program, and contribute to leukemogenesis. This is exemplified by the retroviral oncogene v-myb of the Avian Myeloblastosis Virus (AMV). The v-Myb oncoprotein has gathered mutations that abrogate the balancing function between proliferation and differentiation, that block cell maturation, and that abrogate interaction with C/EBP. As a

result, v-myb transformed progenitors remain immature myeloblasts which maintain their proliferation capacity, and which fail to differentiate thereby causing leukemia (Figure 2).

Chromatin remodeling and lineage specific gene expression

Prerequisites for gene activation in eukaryotic cells are both, to overcome the repressive effects of chromatin and to instruct polymerase II to start transcription. C/EBP α and - β interact with the chromatin remodeling SWI/SNF complex and with 'Mediator'. The interaction between SWI/SNF and C/EBP is required to modify chromatin in such a way that a previously silent gene may become accessible to the activation machinery. We have shown that C/EBP α and - β have specific domains that bind to and recruit the SWI/SNF complex to myeloid loci. SWI/SNF recruitment, together with other transcription factors, such as Myb in the hematopoietic system, is essential for the activation of myeloid differentiation genes. C/EBPs also collaborate with other transcription factors such as PPAR γ during adipogenesis. Similar as in hematopoiesis, C/EBPs recruit the SWI/SNF complex to activate transcription of adipose or fat cell genes. Moreover, interaction between SWI/SNF and C/EBP α is also important for the antiproliferative effect of C/EBP α . Since C/EBPs participate in many cell specification events, recruitment of SWI/SNF could therefore represent a more general switch for cell lineage commitment, terminal differentiation, and proliferation arrest.

Mediator complex: A connection between Ras signaling and C/EBP β activation

Once the gene and its chromatin is 'poised' for expression, the transcription machinery needs to be recruited. Our results show that the activity of C/EBP β is conditional in that C/EBP β acts as a repressor in the absence of signaling and is turned into an activator by signaling through the Ras/MAPK pathway. Upon Ras signaling C/EBP β becomes phosphorylated at a repressor domain. Phosphorylation is accompanied by a conformational change in the protein which abolishes repression and stimulates the transactivational potential of C/EBP β . We have found that active and repressed C/EBP β interacts with two different types of evolutionary conserved multi-subunit complexes that have been termed "Mediator" and that connect transcription factors with the basic transcription machinery, including polymerase II. C/EBP β binds to a subunit of Mediator that was initially identified as a protein that connects between Ras signaling and gene regulation in *C. elegans* development. In its repressive form, C/EBP β thus preferentially binds to a repressive form of Mediator whereas oncoge-

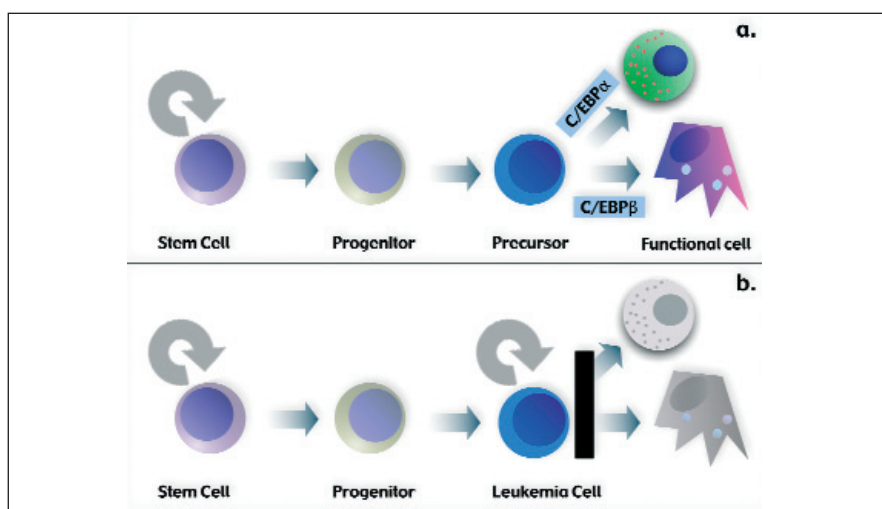


Fig. 2: Function of a hematopoietic oncogene in the development of leukemia. Transcription factors direct cells into particular cell lineages at bifurcation points during hematopoietic differentiation. The function of such critical transcription factors may be altered by mutations (black road block in b). As a consequence cells can no longer undergo proper differentiation, as in a., but maintain self renewal or proliferation capacity and become leukemic cells. Such cells may flood the peripheral blood and kill the host.

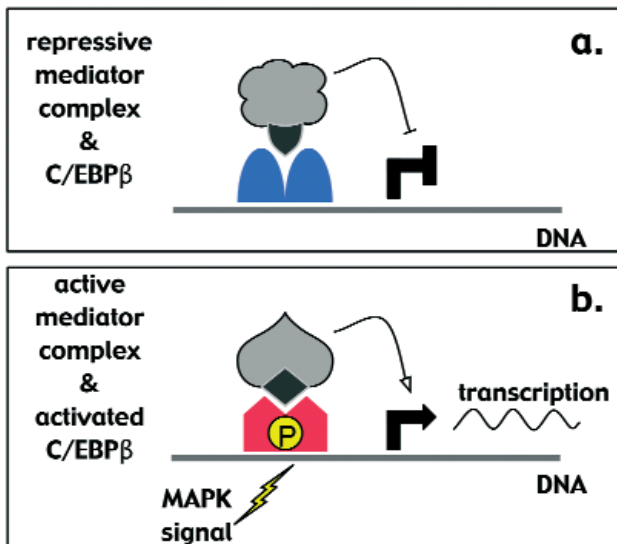


Fig. 3: Model of the interaction between C/EBP β and Mediator.
a. In its repressive state C/EBP β (blue symbols) binds to cis-regulatory sites on DNA and recruits repressive Mediator Complex through the Sur2 subunit.
b. Signaling through the MAP-Kinase pathway leads to phosphorylation of C/EBP β which induces a conformational change. It is thought that this conformational change is allosterically transmitted to Sur2 and further to the Mediator complex that sheds a repressive submodule and becomes active mediator. Active Mediator interacts with polymerase II that transcribes the gene.

nic Ras signaling modifies C/EBP β to select the transcriptionally active Mediator complex, which also associates with RNA polymerase II. This suggests that a Ras-induced structural alteration of C/EBP β determines differential gene activation through a selective interaction with distinct Mediator complexes (Figure 3).

Translational regulation of hematopoietic transcription factors

Several hematopoietic transcription factors are expressed as multiple protein isoforms which arise by alternative initiation of translation at distinct start sites. The resulting transcription factor isoforms have distinct N-terminal domains that may recruit different co-factors with distinct functions for differential gene regulation. Hence, regulation of translation initiation may play an essential role in the control of cell fate. We have shown this to be the case with C/EBP α , - β , and the stem cell and T-cell leukemia transcription factor Scl/Tal1. Moreover, functional experiments showed that isoforms of these transcription factors display specific functions in proliferation control, in the activation of hematopoietic lineage specific genes, and in differentiation. These findings suggest an important role of translational initiation control in both hematopoiesis, and leukemogenesis.

Alternative initiation of translation of C/EBPs and Scl/Tal1 may also be prompted by small upstream open reading frames (uORF). Such uORFs perceive the activity of the translation initiation machinery which is sensitive to environmental inputs such as stress, nutrition, or hormonal changes. The described mechanism of alternative translational initiation seems to be an ancient means of gene regulation since it is already found in phylogenetically "older" yeast systems and allows cells to rapidly adjust genetic programs to environmental changes. Pro-

teins involved in translational control include PI3-kinase, AKT-kinase, PTEN-phosphatase or translation initiation factors eIF-2a or eIF-4E, many of which can also turn into oncogenes. It is very likely that pathways and factors involved in the control of alternative translational initiation may play a far more important role than previously recognized. It is also expected that translational control pathways entail novel targets for innovative drug therapies. Accordingly, we are developing screening systems to discover appropriate drugs.

Transcription co-factors

Transcription factors interact with other trans-regulatory proteins such as co-activators, co-repressors, chromatin remodeling factors, and/or bridging factors for gene regulatory complexes. These proteins provide "missing links" in understanding the complexity of gene regulation and epigenetic modification. Although substantial progress has been made to unravel many of such transcriptional co-factors and complexes, many cofactor interactions and mechanism of gene control are yet to be uncovered. We are therefore searching for proteins that interact with hematopoietic transcription factors and oncoproteins by the "yeast-two-hybrid system" and by co-purification of transcription factor-associated proteins. A number of interesting proteins have already been identified, e.g., proteins that harbor domains implicated in chromatin regulation during embryonal development or proteins with catalytic activity implied in the post-translational modification of the transcriptional apparatus or the chromatin. We are developing murine gene knock-out and gene knock-in mutants as well as RNAi strategies to determine the effects of C/EBPs defective for interactions with these co-factors. Initial experiments have already revealed redundant and essential functions of C/EBP α and C/EBP β during early embryogenesis and neonatal live.

Credits

This manuscript contains a synopsis of the science performed in the last 5 years in my lab at the MDC. I would like to thank all post-doctoral and doctoral scientists and technicians in my lab for their contribution. Above all, my deepest thanks to my dear wife and scientific collaborator, Elisabeth Kowenz-Leutz. Elisabeth has started and participated in many projects as mentioned in the manuscript. I am indebted to Valerie Begay for setting up mouse genetics and C/EBP double KOs, Cornelis F. Calkhoven for his work on translational control, Xianming Mo for his work on mediator, and Christine Müller for her work on proliferation control. I thank Katharina Ahrens for corrections on the manuscript.

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