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# Mechanisms Controlling Hematopoiesis

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## 1. Introduction

Hematopoiesis – the generation of blood cells that proceeds mainly in the bone marrow - is a well-controlled process constantly occurring throughout the life of the mammalian organism. Generally, blood cells are relatively short-lived cells with a life span ranging from few hours to several weeks causing the need for a sustained replenishment of functional erythroid, lymphoid and myeloid cells. The development of mature hematopoietic cells in a hierarchical manner from a pluripotent hematopoietic stem cell over multipotent progenitors that further develop to oligopotent and then to lineage-restricted progenitors requires several control mechanisms at different levels. Transcription factors important for the expression of lineage-specific genes play a major role in the regulation of hematopoietic stem cell maintenance as well as hematopoietic lineage decision. Moreover, the discovery of so-called master transcription factors determining the fate of a terminally differentiated cell population indicates on one side the coordinated processes of hematopoietic cell differentiation but on the other side the complex mechanisms of transcriptional activation and/or repression of specific genes. However, what in turn regulates the expression of transcription factors that finally determine the lineage and differentiation choice of a certain progenitor or immature cell? Is the development into one or another cell type a definitive event or is there some plasticity observed? Which factors are necessary and which sufficient for hematopoietic cell differentiation? These and several other important questions concerning the regulation of development and differentiation of blood cells will be discussed. This chapter summarizes the current knowledge about cell intrinsic, environmental as well as epigenetic mechanisms involved in the control of hematopoiesis under homeostatic as well as infectious conditions.

### 1.1 Hematopoiesis

The hematopoietic system is traditionally categorized into two separate lineages, the lymphoid lineage responsible for adaptive immunity and the myeloid lineage embracing morphologically, phenotypically and functionally distinct cell types like innate immune cells as well as erythrocytes and platelets. Mature hematopoietic cells, except some rare lymphoid cell types, are relatively short-lived with life spans ranging from few hours for granulocytes to a couple of weeks for erythrocytes demanding a continued replenishment of functional cells. This process is named hematopoiesis and takes place primarily in the bone marrow, where few hematopoietic stem cells give rise to a differentiated progeny following a series of more or less well-defined steps of multipotent progenitors and lineage-restricted

precursors leading to a hierarchical structure of the process. During the course of hematopoiesis cells lose their proliferative potential as well as multi-lineage differentiation capacity and progressively acquire characteristics of terminally differentiated mature cells.

## 1.2 Hematopoietic stem cells

In the hematopoietic differentiation hierarchy, the most primitive cells with highest multipotent activity are long-term repopulating hematopoietic stem cells (LT-HSC). One of the first definitions of true HSC meaning LT-HSC came from bone marrow transplantation experiments in mice determining HSC by their capacity to reconstitute several times the hematopoietic system of lethally irradiated adult organisms. Such experiments have demonstrated that HSC possess multi-potentiality as well as the ability to produce exact replicas upon cell division, named self-renewal capacity. In contrast to real HSC, short-term repopulating HSC (ST-HSC) defined by their ability to contribute transiently to the production of lymphoid and myeloid cells in lethally irradiated recipients, are often described misleadingly as self-renewing cells. The contemporary model of hematopoietic stem cells proposes the affiliation of ST-HSC to the group of multipotent progenitors (MPP), which are characterized by a more limited proliferative potential, but retained ability to differentiate into various hematopoietic lineages (Kondo et al., 2003; Weissman & Shizuru, 2008). Concerning MPP hierarchy, a defined model is not available at the moment, because several studies have demonstrated different types of multipotent progenitors with myelo-lymphoid or myelo-erythroid potential, such as the lymphoid-primed multipotent progenitor (LMPP) (Iwasaki & Akashi, 2007).

Additionally, a lot of research concerning prospective isolation and characterization of HSC and multipotent progenitors has provided insight into the surface marker expression on these types of cells leading to the definition of HSC and multipotent progenitors as cells being mainly negative for lineage markers but positive for the surface markers Sca1 and Kit. This fraction of bone marrow cells is also named LSK-fraction (Lin<sup>-</sup>Sca1<sup>+</sup>Kit<sup>+</sup>) and comprises all stem cell capacity of the hematopoietic system, whereby HSC are defined as Lin<sup>-</sup>Kit<sup>+</sup>Sca1<sup>+</sup>Flt3<sup>-</sup> and MPP as Lin<sup>-</sup>Kit<sup>+</sup>Sca1<sup>+</sup>Flt3<sup>+</sup>. Furthermore, the Slam (signaling lymphocyte activation molecule) family receptors CD150 and CD48 are useful surface markers allowing to distinguish inside the LSK-fraction between HSC (CD150<sup>+</sup>CD48<sup>-</sup>) and multipotent progenitors (CD150<sup>-</sup>CD48<sup>-</sup>) as well as the most restricted progenitors (CD48<sup>+</sup>) (Kiel et al., 2005).

Under homeostatic conditions, the number of HSC remains relatively constant and the majority of HSC stays in a quiescent state that contributes not only to their long-term maintenance, but also allows a rapid cell cycle entry upon a variety of differentiation cues. The minority of HSC is in an active and dividing state and gives rise to all hematopoietic cells meaning that these few active HSC not only have to self-renew, but also have to produce all differentiated progeny. These different cell fates can only be achieved by an asymmetric division of the HSC, which allows the generation of two non-identical daughter cells, one maintaining stem cell identity and the other becoming a differentiated cell. Two different mechanisms are proposed by which asymmetry could be achieved: first by divisional asymmetry that is introduced by unequally redistributed cell-fate determinants in the cytoplasm (Florian & Geiger, 2010). An alternative possibility would be the environmental asymmetry, which is caused by different extrinsic signals provided by

distinct local microenvironments and provokes different cell fate decisions of two identical daughter cells (Wilson, A. & Trumpp, 2006).

### 1.3 Lineage-committed progenitors

Downstream of the HSC and MPP populations with high proliferative and self-renewal capacity starts the differentiation process in hematopoiesis leading to oligopotent and later on to lineage-committed progenitors with a diminished proliferation but increased differentiation. The contemporary model of hematopoiesis (Figure 1) assumes that the decision for differentiation into the lymphoid/myeloid or megakaryocyte/erythrocyte lineages probably occurs very early in hematopoiesis. Several studies have demonstrated that multipotent progenitors like the lymphoid-primed multipotent progenitor (LMPP, Lin<sup>-</sup>Kit<sup>+</sup>Sca1<sup>+</sup>CD150<sup>-</sup>CD34<sup>+</sup>Flt3<sup>hi</sup>) retain only minor megakaryocyte/erythrocyte lineage potential, whereas the vast majority of progenitors appears to be committed to the granulocyte/monocyte as well as the lymphoid lineage (Iwasaki & Akashi, 2007).

In the next step of ongoing differentiation oligopotent progenitors with differentiation capacity for several hematopoietic lineages develop from an ancestor, the common lymphoid progenitor (CLP) (Kondo et al., 1997) and the common myeloid progenitor (CMP) (Akashi et al., 2000). The CLP is the earliest population in the lineage-negative fraction that upregulates the receptor for interleukin 7 (IL-7), an essential cytokine for T and B cell development. Furthermore, the CLP carries differentiation potential for all types of lymphoid cells including B cells, T cells and NK cells. The surface marker profile of CLP is defined as Lin<sup>-</sup>Sca1<sup>lo</sup>Kit<sup>lo</sup>IL7R $\alpha$ <sup>+</sup> (Kondo et al., 1997). In contrast to CLP, the CMP resides in the Lin<sup>-</sup>Sca1<sup>-</sup>Kit<sup>+</sup> population in bone marrow that can be further fractioned by expression of the Fc $\gamma$  receptor II/III (Fc $\gamma$ RII/III) and CD34 leading to three distinct progenitor populations.

The CMP is defined as Fc $\gamma$ RII/III<sup>lo</sup>CD34<sup>+</sup> and can give rise to all types of myeloid colonies in clonogenic assays, while the Fc $\gamma$ RII/III<sup>hi</sup>CD34<sup>+</sup> granulocyte-monocyte progenitor (GMP) is restricted to granulocytes and macrophages. The Fc $\gamma$ RII/III<sup>lo</sup>CD34<sup>-</sup> megakaryocyte-erythrocyte progenitor (MEP) is delimited to megakaryocytes and erythrocytes (Akashi et al., 2000).

Still a matter of dispute is the dendritic cell (DC) development, because DC mainly are the progeny of GMP, but can also be generated from lymphoid progenitors such as CLP and pro T cells under certain conditions (Manz et al., 2001). However, the majority of plasmacytoid DC (pDC) and conventional or myeloid DC (mDC) develop successively by several commitment steps downstream of the GMP in the bone marrow. The first step is the development of the monocyte/macrophage and DC precursor (MDP) (Fogg et al., 2006) (MDP) out of the GMP that has lost differentiation potential for granulocytes and expresses the Fc $\gamma$ RII/III and CD34 at a comparable level to the GMP, but is also Kit<sup>lo</sup>CX<sub>3</sub>CR1<sup>+</sup>. Further differentiation of MDP, which is accompanied by the loss of monocyte potential, leads to the common DC precursor (CDP) defined as Lin<sup>-</sup>Kit<sup>int</sup>Flt3<sup>+</sup>M-CSFR<sup>+</sup> population that can only give rise to pDC and mDC (Geissmann et al., 2010; Naik et al., 2007; Onai et al., 2007).

Besides the characterization of MDP and CDP by several studies, further progenitor populations for eosinophils, basophils and mast cells have been isolated downstream of the GMP and their position in the hematopoietic hierarchy is depicted in Figure 1. Moreover,

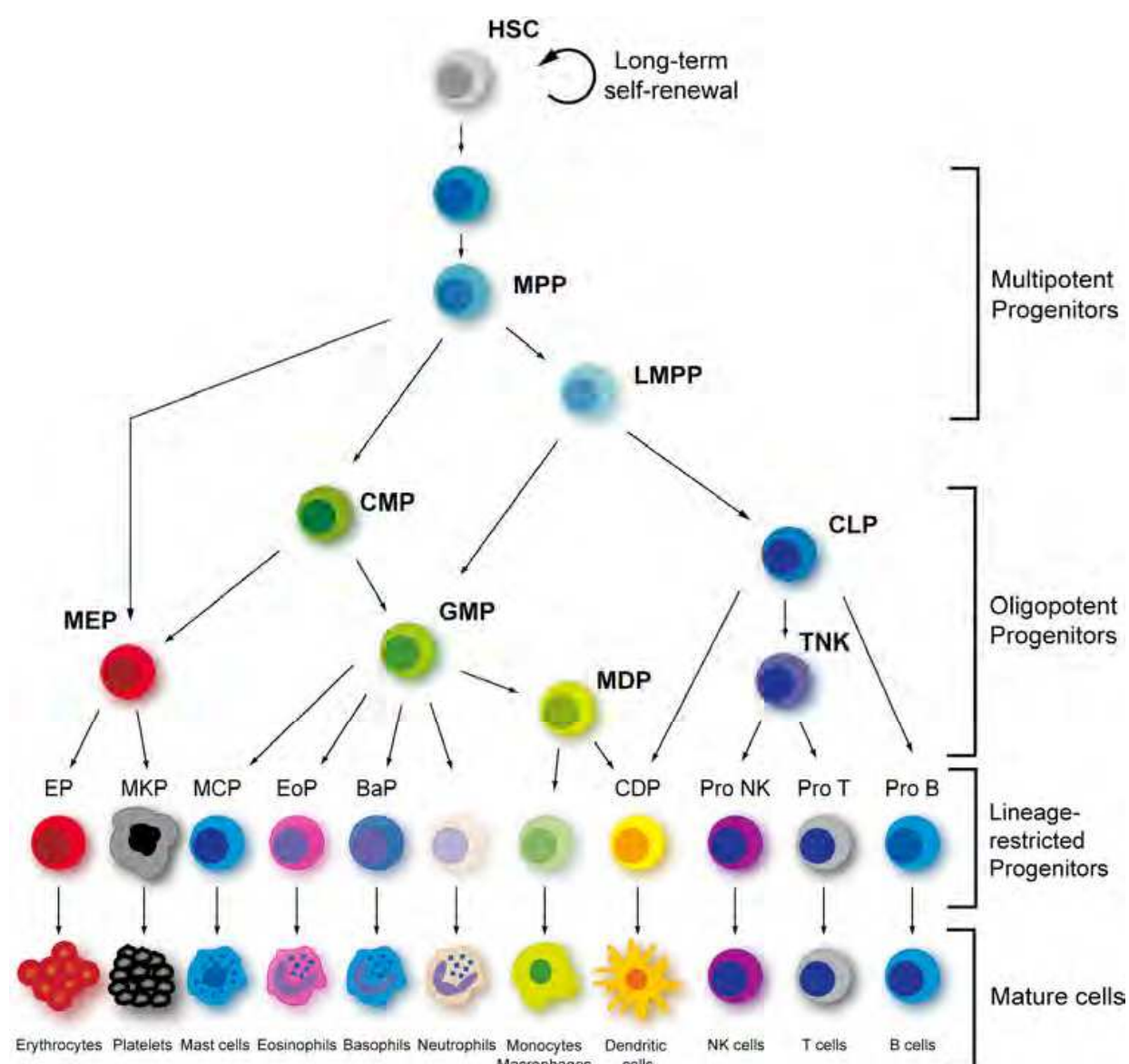


Fig. 1. Model of the hematopoietic hierarchy in the mouse.

The developmental course shown in the scheme is proposed using results generated by prospective isolation and characterization of different progenitors. HSC, hematopoietic stem cell; MPP, multipotent progenitor; LMPP, lymphoid-primed multipotent progenitor; CLP, common lymphoid progenitor; CMP, common myeloid progenitor; MEP, megakaryocyte-erythrocyte progenitor; GMP, granulocyte-macrophage progenitor; MDP, monocyte-dendritic cell progenitor; TNK, T cell NK cell progenitor; EP, erythroid progenitor; MKP, megakaryocyte progenitor; MCP, mast cell progenitor; EoP, eosinophil progenitor; BaP, basophil progenitor; CDP, common dendritic cell progenitor.

the monopotent megakaryocyte lineage-committed progenitor (MKP) (Pronk et al., 2007) and erythroid progenitor (EP) (Terszowski et al., 2005) have been described downstream of the MEP. Only for the monocyte/macrophage lineage and the neutrophil granulocytes, a putative committed precursor downstream of the GMP has not been identified to date (Iwasaki & Akashi, 2007). With regard to lymphoid development one committed precursor downstream of the CLP is the bipotent T/NK cell progenitor that resides in the



bone marrow and is able to generate thymic- and bone marrow-dependent NK cells as well as T cells (Nozad Charoudeh et al., 2010).

## 1.4 Factors involved in the regulation of hematopoiesis

The highly regulated differentiation process of quiescent HSC towards different progeny of mature hematopoietic cells is associated with a variety of cell fate choices at every single step of hematopoiesis. These different choices comprise quiescence, self-renewal or differentiation at HSC level as well as proliferation, lineage commitment and terminal differentiation at the progenitor or precursor level. Of course, different cell fate choices require at each step in the hematopoietic hierarchy a process of decision-making that is presumed to be dependent on and regulated by a combination of intrinsic factors that embrace lineage-determining transcription factors and their epigenetic regulation as well as extrinsic regulators such as cytokines.

### 1.4.1 Maintenance of HSC characteristics

For the maintenance of HSC with respect to quiescence, self-renewal and suppression of differentiation, the major intrinsic factors belong to the Bmi1-p53 axis of cell cycle regulators and the PI3K signaling pathway. Bmi1 is a member of the Polycomb group gene family that controls cell proliferation via repression of the *Ink4/Arf* locus. Therefore, Bmi1 supports self-renewal by suppressing transcription of the cell cycle inhibitors p16<sup>Ink4a</sup> and p19<sup>ARF</sup>, which are encoded in the *Ink4/Arf* locus, whereas the tumor suppressor p53 contributes to the regulation of HSC quiescence via inhibition of cell cycle (Warr et al., 2011). In contrast, the PI3K signaling pathway controls cell proliferation, growth and survival via integration of numerous upstream signals, including growth factors, nutrients and oxygen status.

Additionally, several extrinsic factors have been identified that are necessary for preservation of HSC stemness. The extrinsic regulators embrace soluble membrane-bound extrinsic factors including cytokines (fms-related tyrosine kinase 3-ligand, stem cell factor), chemokines (CXCL12) and growth factors (Angiopoietin-1, granulocyte-CSF, granulocyte-macrophage-CSF), as well as Wnt (wingless type), Notch, Hedgehog and the TGF $\beta$  (transforming growth factor  $\beta$ ) family of cytokines. These extrinsic factors are provided by a specialized microenvironment in the bone marrow, the so-called stem cell niche that resides in the endosteal and vascular compartments of the bone. In these areas, the bone marrow cells of hematopoietic and non-hematopoietic origin like megakaryocytes, osteoblasts, endothelial cells and CXCL12-abundant reticular (CAR) cells create a supportive microenvironment via physical interaction with HSC and production of soluble factors (Warr et al., 2011).

### 1.4.2 Transcription factors involved in lineage commitment

At the cellular level the differentiation process from HSC into lineage-committed hematopoietic cells involves the selective activation of lineage-specific genes as well as the silencing of lineage-foreign genes and developmental regulators in a defined order. The orchestration of such complex lineage-determining programs is dependent on several factors, but extensive research has emphasized the essential role of gene regulatory networks in directing cell fate choice and lineage restriction. These gene regulatory

networks are composed of several master transcription factors that join special features, such as mutual regulation of transcriptional activity by antagonism as well as lineage-determining functions via activation of lineage-specific genes and repression of lineage-foreign genes. The first example pointing out the importance of such transcription factors is the transition from self-renewing HSC towards more committed MPP that is dependent on the transcription factor CCAAT-enhancer binding protein  $\alpha$  (C/EBP $\alpha$ ). The prototype of the C/EBP family displays all characteristic features of the transcription factor family, such as the N-terminal transactivation domain as well as the C-terminal DNA-binding domain consisting of a highly conserved basic region and a leucine zipper dimerization domain. Prerequisite for binding of C/EBP $\alpha$  to the cognate DNA-site is the homo- or heterodimerization with another transcription factor via the leucine zipper domain that in turn allows the basic region to bind to the CCAAT motif (Johnson, 2005; Lekstrom-Himes, J. & Xanthopoulos, 1998). Evidences for the function of C/EBP $\alpha$  in hematopoietic differentiation revealed from studies on conditional C/EBP $\alpha$ -deficient mice, which demonstrated a competitive advantage of C/EBP $\alpha$ -deficient HSC over wild type HSC in reconstitution experiments. Further analyses of the transcriptome of C/EBP $\alpha$ -deficient HSC have confirmed that the expression of the self-renewal factor Bmi1 is increased in these cells, suggesting C/EBP $\alpha$  as a pro-differentiation factor in HSC fate decision (Zhang et al., 2004).

#### 1.4.2.1 Erythroid-megakaryocyte lineage commitment

Probably, the next step in decision-making during differentiation is the choice for erythroid versus myeloid-lymphoid lineage restriction at the transition from MPP to LMPP or MEP that is regulated by the E-twenty six (Ets) family transcription factor PU.1 and the transcription factor GATA-binding protein 1 (GATA-1). GATA-1 is expressed in erythroid, megakaryocyte and mast cell as well as eosinophil lineages and contains zinc fingers, which mediate DNA binding to the WGATAR DNA sequence as well as protein-protein interaction (Bresnick et al., 2010; Morceau et al., 2004). In contrast to GATA-1, PU.1 is restricted to monocyte as well as B lymphoid lineages and consists of a N-terminal transactivation domain, a PEST-domain (proline, glutamic acid, serine and threonine rich sequence) and the eponymous Ets-domain at the C-terminus, which mediate DNA binding to an 11 bp sequence with a central GGAA motif (Gangenahalli et al., 2005; Sharrocks, 2001). Additionally, both transcription factors are detectable in MPP and gene disruption studies have demonstrated the indispensable functions of GATA-1 and PU.1 for megakaryocyte/erythrocyte and myeloid/lymphoid development, respectively. Analyses of systemic PU.1-deficient mice revealed a complete loss of CMP, GMP and CLP populations but normal numbers of MEP causing impaired lymphoid and myeloid cell development as well as retained megakaryocyte/erythrocyte development (Scott et al., 1994). In contrast, GATA-1-deficient mice die between embryonic day 10.5 and 11.5 due to severe anemia resulting from a maturation arrest of erythroid cells (Fujiwara et al., 1996). Further support for the lineage instructive role of GATA-1 originated from the forced expression of GATA-1 in lineage-committed progenitors like GMP and CLP that exclusively leads to megakaryocyte/erythrocyte development (Iwasaki et al., 2003). Several other studies dealing with certain aspects of the molecular interaction of PU.1 and GATA-1 as well as their gene regulatory capacity revealed the cross-antagonism between these proteins involving direct physical interaction of both factors that results in an inhibition of the transactivation potential of the counterpart (Laslo et al., 2008). Based on these findings,

GATA-1 is prospected as the erythrocyte/megakaryocyte lineage determinant, whereas PU.1 is regarded as the myeloid/lymphoid lineage determinant. Regarding the regulation of erythrocyte versus megakaryocyte development, the detailed molecular mechanisms are not fully understood, but several transcription factors involved in this process are described such as Friend of GATA-1 (FOG-1), Fli-1 or Krueppel-like factor 1 (KLF1) (Kerenyi & Orkin, 2010; Szalai et al., 2006).

#### 1.4.2.2 Myeloid lineage commitment

Downstream of LMPP, lineage choice embraces myeloid, as well as B or T lymphoid lineage and mainly depends on the transcription factors PU.1, early B cell factor 1 (EBF1) and Notch. For myeloid lineage restriction, a high expression level of PU.1 is necessary, whereas low levels of PU.1 plus EBF1 expression establish the B lymphoid lineage restriction and Notch instructs the T lymphoid lineage choice. Regarding granulocyte and monocyte development, besides PU.1, the transcription factor C/EBP $\alpha$  has to be enumerated. Studies have demonstrated that conditional deletion of C/EBP $\alpha$  in bone marrow cells of mice using the Mx1-Cre system leads to a total lack of mature granulocytes and a partial lack of monocytes due to a differentiation block at the transition from CMP to GMP (Zhang et al., 2004). Moreover, lineage choice between monocytes and granulocytes depends on the expression level of PU.1 and C/EBP $\alpha$ , which has been shown by studies using different mouse as well as *in vitro* models for diminished PU.1 expression in the hematopoietic system. In all experimental setups, reduced expression of PU.1 is followed by an augmented granulopoiesis to the disadvantage of monopoiesis. Additionally, gene expression analyses of PU.1-deficient progenitors have demonstrated a decreased or even absent expression of several monocyte-specific genes, like the macrophage scavenger receptor or the M-CSF receptor. Furthermore, the need for C/EBP $\alpha$  during the transition from CMP to GMP is possibly due to the transcriptional upregulation of PU.1, since forced C/EBP $\alpha$  expression in hematopoietic progenitors favors monopoiesis and not granulopoiesis, whereas exogenous C/EBP $\alpha$  in myeloid cell lines directs granulopoiesis (Friedman, 2007). Nevertheless, C/EBP $\alpha$  is probably indispensable for granulocyte development due to the transcriptional upregulation of several granulocyte-specific factors. One of these factors is the transcriptional repressor growth factor independent 1 (Gfi1), which is necessary for the repression of proliferation and of monocyte lineage-promoting factors such as M-CSF (Borregaard, 2010). Another important target of C/EBP $\alpha$  is the transcription factor C/EBP $\epsilon$  that is important for terminal granulocyte differentiation, because of the transcriptional control of granule-specific genes (lactoferrin and gelatinase) as well as genes necessary for cell cycle regulation (Borregaard, 2010).

Besides the upregulation of other transcription factors, C/EBP $\alpha$  forces granulocyte development additionally by transactivation of various genes, such as G-CSF receptor (Hohaus et al., 1995; Smith, L. T. et al., 1996) or myeloperoxidase (MPO) (Wang, W. et al., 2001), and downregulation of proliferation by direct interaction with the cell cycle regulator E2F (D'Alo et al., 2003; Theilgaard-Monch et al., 2005). In line with these experimental results is the association of inactivating C/EBP $\alpha$  mutations with hematopoietic malignancies like acute myeloid leukemia and high-risk myelodysplastic syndrome proposing that C/EBP $\alpha$  possesses the ability to arrest cell proliferation and to drive terminal differentiation (Koschmieder et al., 2009). Taken together, the plethora of studies implicates the following model for monocyte versus granulocyte lineage choice: First



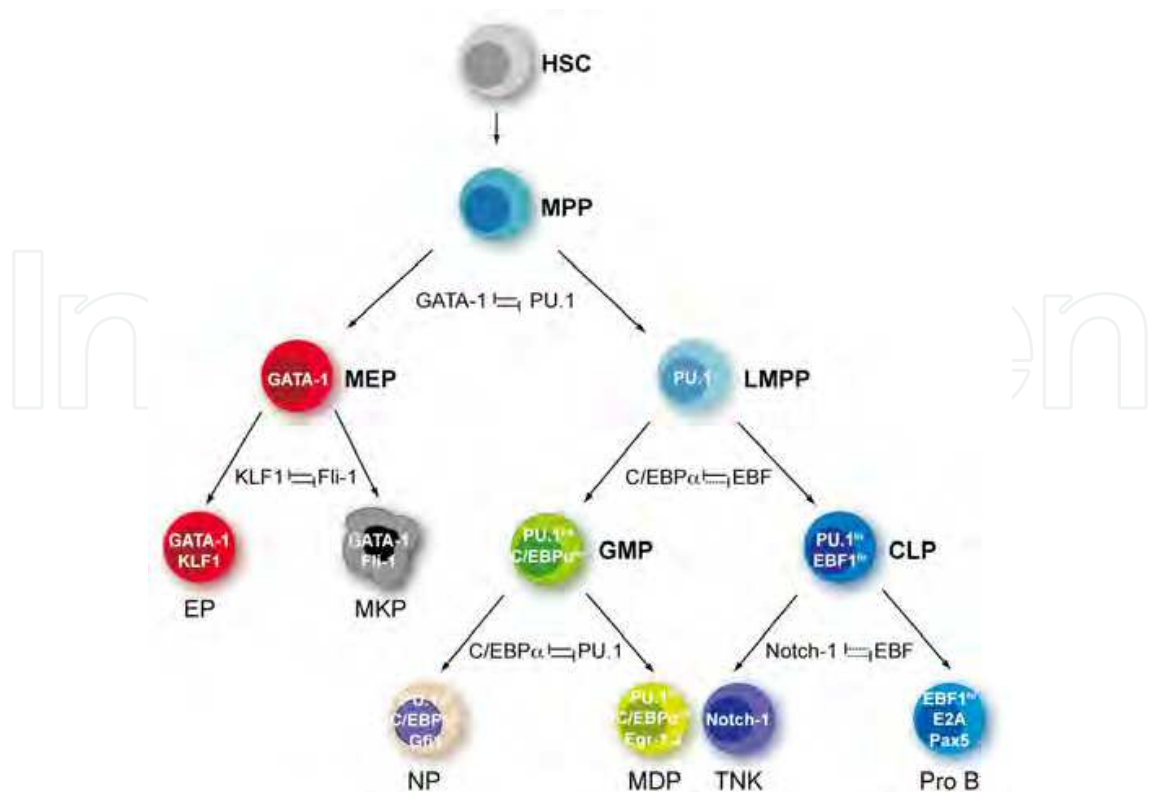


Fig. 2. Transcription factor network regulating lineage commitment. The scheme displays a simplified overview of gene regulatory networks, which have a major influence on hematopoietic lineage choice during hematopoiesis. Supposed (dashed lines) and proved (continuous lines) cross-antagonisms between key transcription factors which function to regulate binary cell fate choices are noted in the scheme. Additionally, transcription factors that are important for the generation of particular intermediates are noted in white. HSC, hematopoietic stem cell; MPP, multipotent progenitor; LMPP, lymphoid-primed multipotent progenitor; CLP, common lymphoid progenitor; CMP, common myeloid progenitor; MEP, megakaryocyte-erythrocyte progenitor; GMP, granulocyte-macrophage progenitor; NP, neutrophil progenitor; MDP, monocyte-dendritic cell progenitor; TNK, T cell NK cell progenitor; EP, erythroid progenitor; MKP, megakaryocyte progenitor.

of all, C/EBPα is needed for the transition from CMP to GMP by induction of PU.1 expression. High protein levels of PU.1 induce monopoiesis via interaction with other transcription factors like interferon regulatory factor 8 (IRF8) or activating protein-1 family transcription factors (AP-1/Jun proteins) and the transcriptional activation of monocyte-specific genes (Friedman, 2007). However, AP-1 family transcription factors are also able to heterodimerize with C/EBPα (Cai et al., 2008) implicating an inhibition mechanism of PU.1 for granulocyte development by sequestering the binding partners of C/EBPα. In contrast to the high protein levels of PU.1 that favor monopoiesis, insufficient activation of PU.1 transcription allows C/EBPα to induce the granulopoiesis program accompanied by suppression of monopoiesis (Figure 2).

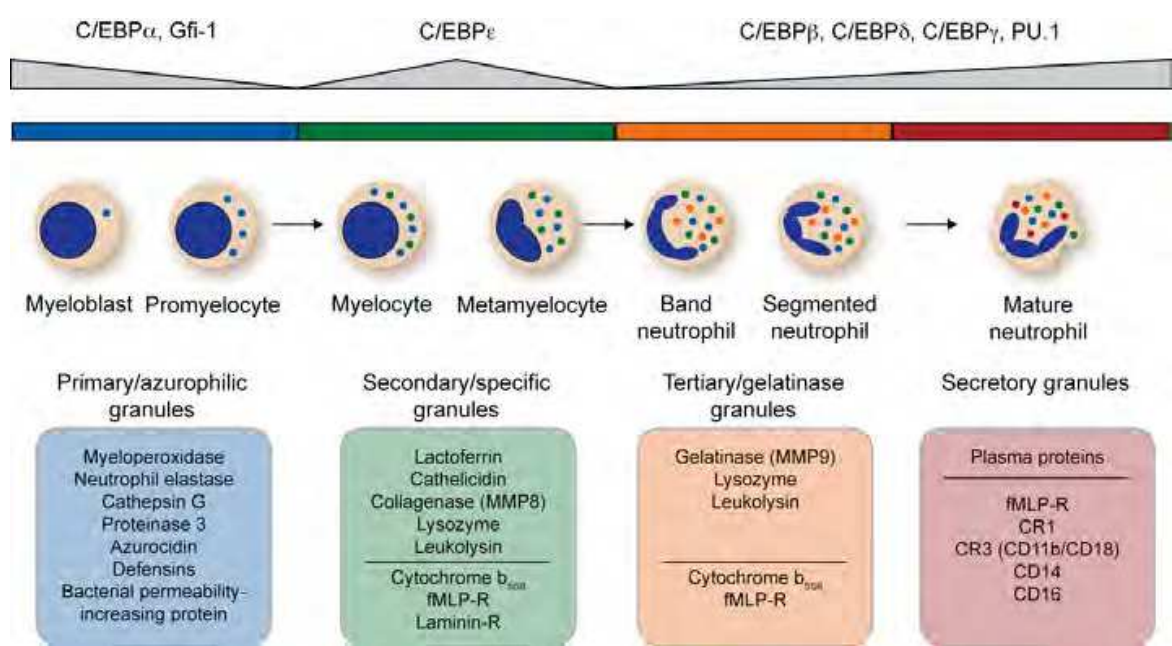
Terminal granulopoiesis starts with the myeloblast and promyelocyte state, where the switch from proliferation to differentiation takes place, displayed by the loss of ability for

cell division after the promyelocyte state. Moreover, the formation of the first granules starts, which are named primary or azurophilic granules. The most important transcription factors at myeloblast/promyelocyte stage are C/EBP $\alpha$  and Gfi1, which are necessary for the suppression of monocyte development and proliferation as well as for the transcriptional activation of granulocyte-specific genes like *MPO*, *ELANE* or *CEBPE* (Borregaard, 2010; Koschmieder et al., 2009; Theilgaard-Monch et al., 2005). The importance of Gfi1 and *ELANE* has been demonstrated by studies analyzing the genetic background of severe congenital neutropenia (SCN) and other forms of neutropenia. These studies revealed that one of the major causes for loss of neutrophil differentiation beyond promyelocyte state are mutations in the *ELANE* gene (Dale et al., 2000; Horwitz et al., 1999), but in rare cases of SCN also mutations of the *GFI1* gene have been described (Person et al., 2003). Detailed analyses of Gfi1 in mice further supported the function of Gfi1 as molecular switch towards granulocyte development by suppression of monocyte-specific genes, like *Csf1* (M-CSF) and *Csf1r* (M-CSFR) (Zarebski et al., 2008).

Ongoing differentiation beyond promyelocytes leads to the development of myelocytes and metamyelocytes, which are defined by the beginning of nuclear segmentation and the appearance of secondary (also called specific) granules as well as the exit from cell cycle. The regulation of secondary granule protein expression and the exit from cell cycle mainly depends on the transcription factor C/EBP $\epsilon$ , whose expression peaks in myelocytes and metamyelocytes (Bjerregaard et al., 2003; Theilgaard-Monch et al., 2005). Based on studies using C/EBP $\epsilon$ -deficient mice, which displayed neutrophil-specific defects including bilobed nuclei, abnormal respiratory burst and compromised bactericidal activity as well as impaired chemotaxis (Lekstrom-Himes, J. & Xanthopoulos, 1999; Yamanaka et al., 1997), the genetic cause of a very rare congenital disorder named neutrophil specific granule deficiency (SGD) has been delineated to the *CEBPE* locus (Lekstrom-Himes, J. A. et al., 1999). Additional studies have revealed the essential functions of C/EBP $\epsilon$  for the expression of secondary and tertiary granule proteins (Verbeek et al., 1999; Yamanaka et al., 1997) and demonstrated the direct interaction of C/EBP $\epsilon$  with E2F1 and Rb protein, finally leading to cell cycle exit (Gery et al., 2004).

The last step of terminal granulopoiesis, the differentiation into band and segmented neutrophils leads to mature neutrophils with finally segmented nuclei and tertiary as well as secretory granules. In the course of neutrophil terminal differentiation, C/EBP $\alpha$  expression gradually diminishes during the myeloblast stage. C/EBP $\epsilon$  peaks at the myelocyte/metamyelocyte stage, whereas the expression level of the transcription factors PU.1, C/EBP $\beta$ , C/EBP $\delta$  and C/EBP $\gamma$  increases subsequently to the metamyelocyte stage (Bjerregaard et al., 2003). However, gene deletion studies using C/EBP $\beta$ - or C/EBP $\delta$ -deficient mice revealed no hematopoietic abnormalities with regard to terminal granulopoiesis. Still, Hirai and colleagues have demonstrated the indispensable role of C/EBP $\beta$  during emergency granulopoiesis in response to cytokine treatment or fungal infection in contrast to C/EBP $\alpha$  and C/EBP $\epsilon$ , which were not upregulated under these conditions (Hirai et al., 2006). In the case of the transcription factor PU.1, a conditional gene deletion model has evidenced a PU.1-dependent transcriptional activation of *gp91<sup>phox</sup>*, a component of the NADPH oxidase, as well as of *Mac-1/CD11b* (Dakic et al., 2005) (Figure 3).

Terminal differentiation during monopoiesis leads to monocytes, macrophages as well as dendritic cells and involves again the selection of specific gene expression programs to



**Fig. 3. Terminal granulopoiesis in the bone marrow.**  
The terminal granulopoiesis that is characterized by sequential formation of different granule types and segmentation of the nucleus starts at the myeloblast/promyelocyte stage and ends with mature neutrophils. Granule types not only differ in the time point at which they are formed, but also in their specific content, which is described at the bottom of the figure. Above the line in the boxes matrix content is depicted and beneath the proteins that are located to the vesicle membrane. At different stages of terminal granulopoiesis several transcription factors, which are indicated on top of the figure, are important for the regulation of maturation and timed expression of granule proteins.

determine cell fates. Additionally, several subtypes of macrophages or DC have been described in recent years bringing more complexity into monopoietic differentiation. Nevertheless, some key transcription factors with indispensable functions for monopoiesis are known already. For example, PU.1 is not only required for myeloid lineage commitment, but also for macrophage versus DC lineage choice during late myelopoiesis. Intermediate PU.1 expression at GMP stage results in the activation of the macrophage-specific transcription factors Egr-1 and Egr-2 (Laslo et al., 2006), whereas high expression of PU.1 promotes the induction of DC fate via repression of the macrophage-inducing transcription factors c-Maf and MafB (Bakri et al., 2005). In addition, gain-of-function experiments have demonstrated that ectopic expression of MafB, c-Maf, Egr-1 or IRF8 in early progenitors can drive monocyte or macrophage lineage commitment. In contrast, RelB induces DC differentiation and SpiB pDC differentiation in monocytic intermediates (Auffray et al., 2009; Geissmann et al., 2010). However, the detailed molecular mechanisms driving terminal monopoiesis remains to be elucidated.

1.4.2.3 B cell lineage commitment

B cells develop from CLP in the bone marrow, where several stages of B cell development have been defined. The earliest B lineage precursors are the pre/pro B cells, which begin to express the B lineage specific marker B220 at their surfaces. The transition of pre/pro B cells to the pre B cell stage is characterized by the upregulation of the surface marker CD19 as

well as by the rearrangement of the immunoglobulin (Ig) heavy chain gene locus. Successful rearrangement of the Ig light chain locus is the prerequisite for the development to immature IgM expressing B cells. At this stage the antigen-independent phase of B cell development is almost complete. IgM<sup>+</sup> cells are ready to leave the bone marrow to enter peripheral secondary lymphoid organs where they first develop via the IgM<sup>+</sup>IgD<sup>+</sup> stage to mature IgD<sup>+</sup> B cells. These cells undergo final maturation during the antigen-dependent phase of B cell development.

In addition to cytokines and cytokine receptors, several key transcription factors have been identified necessary for the B lineage commitment as well as for the maintenance of the B cell fate, like Ikaros, Gfi1, PU.1, E2A, EBF1 and Pax5. Prior to the differentiation of CLP, PU.1 is involved in the expression of components of the IL-7 signaling pathways (DeKoter et al., 2007) essential for EBF1-dependent lineage restriction in early lymphoid progenitors (Tsapogas et al., 2011). Additionally, the level of PU.1 expression predicts the decision between the myeloid and the B cell lineage. Low levels of PU.1 favors B cell development whereas high levels promote myeloid cell differentiation (DeKoter & Singh, 2000). The upregulation of the transcriptional repressor Gfi1 was suggested to be responsible for the down-modulation of PU.1 expression in early progenitors by displacing PU.1 from its upstream autoregulatory element and therefore for the promotion of B lineage decision (Spooner et al., 2009). In MPP, Gfi1 is upregulated by Ikaros to antagonize PU.1 expression, thus favoring B cell development (Spooner et al., 2009). CLP begin to express genes associated with committed B cells including E2A as well as EBF1 at the onset of B lymphopoiesis (Roessler et al., 2007; Seet et al., 2004; Smith, E. M. et al., 2002). The deficiency of these factors leads to a block of B cell development at a very early stage, even before D<sub>H</sub>-J<sub>H</sub> rearrangement of the IgH gene (Bain et al., 1994; Lin, H. & Grosschedl, 1995; Zhuang et al., 1994). In contrast, forced expression of E2A and EBF1 revealed that both factors cooperate in the upregulation of several B cell-specific genes, like Pax5, the surrogate light chain  $\lambda 5$  gene, the *VpreB*, *Ig $\alpha$*  and *Ig $\beta$*  genes, plus the genes coding for *Rag1* and *Rag2* (Kee & Murre, 1998; O'Riordan & Grosschedl, 1999; Sigvardsson et al., 1997). In addition, the transcriptional co-activator *Pou2af1* (BOB.1/OBF.1; OCA-B) and the transcription factor *FoxO1* were identified as direct targets of EBF1 (Zandi et al., 2008). In CLP the expression of Pax5 is still low. Consistent with the observation that Pax5 is essential for B lineage commitment, CLP still retain T cell developmental potential. The expression of Pax5 is detectable at the pro B cell where Pax5 antagonizes T cell development by blocking Notch1 (Souabni et al., 2002). Additionally, Pax5 interferes with the developmental potential to differentiate into several other hematopoietic lineages, since in the absence of Pax5 but in the presence of appropriate cytokines pro B cells are able to differentiate *in vitro* into NK cells, dendritic cells, macrophages, granulocytes and osteoclasts (Nutt, S. L. et al., 1999) indicating that the expression E2A and EBF1 is not sufficient to commit B cell progenitors to the B cell lineage in the absence of Pax5. Therefore, Pax5 plays an essential and dual role in B lineage development, it represses non-B cell-specific genes, like the genes coding for the M-CSFR or for MPO (Nutt, S. L. et al., 1999), whereas in the same time it activates the B lineage-specific gene program (Nutt, S. L. et al., 1998; Schebesta et al., 2002). Thus, Pax5 controls the pre-BCR signaling by promoting the V to DJ recombination at the *IgH* locus (Nutt, S. L. et al., 1997) and also by regulating directly the expression of the signaling molecule BLNK (Schebesta et al., 2002). Additionally, Pax5 is essential for the upregulation of *CD19* and *Ig $\alpha$*  gene expression (Kozmik et al., 1992; Nutt, S. L. et al., 1997). Pax5-deficient



B cells are arrested at the pro B cell stage while expressing normal levels of E2A and EBF1 as well as of their target genes (Nutt, S. L. et al., 1998; Nutt, S.L. et al., 1997), indicating that E2A and EBF1 are upstream of Pax5 in the hierarchical order of lineage-determining transcription factor expression.

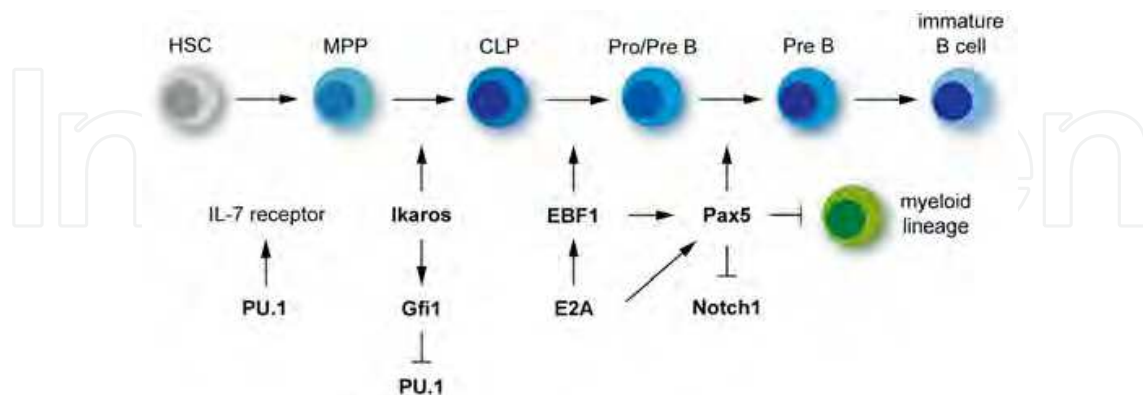


Fig. 4. Key transcription factors involved in B lymphopoiesis.

B cell development is driven by the consecutive activation of lineage-determining transcription factors like E2A, EBF1 and Pax5 and the repression of lineage-foreign genes. Transcription factors are highlighted in bold. HSC, hematopoietic stem cell; MPP, multipotent progenitor; CLP, common lymphoid progenitor.

However, sustained expression of EBF1 in Pax5-deficient hematopoietic precursor cells efficiently blocks myeloid and T lineage potential *in vivo*. Moreover, overexpression of EBF1 in Pax5-deficient pro B cells represses alternative lineage potential indicating that EBF1 promotes the commitment of the B cell lineage independently of Pax5 (Pongubala et al., 2008) (Figure 4). E2A in turn is required for the initiation but also for the maintenance of the expression of EBF1, Pax5 and the B cell-specific gene program at the pro B cell stage (Kwon et al., 2008). E2A exerts its instructive role not only in the bone marrow at the pro and pre B cell stage as well as at the immature B cell stage, but also in peripheral lymphatic organs during the formation of germinal center B cells (Kwon et al., 2008). In contrast, E2A is dispensable for Ig class switch recombination as well as for the generation of mature splenic subpopulations, like marginal zone B cells, follicular B cells and B1 cells. Also, the memory B cell subpopulation and the plasma cell generation is unaffected by the loss of E2A (Kwon et al., 2008).

Conditional inactivation of Pax5 revealed its requirement for the maintenance of B cell identity also during late B cell development in peripheral lymphatic organs (Horcher et al., 2001). Upon exposure to an antigen B lymphocytes can either maintain their B cell identity and differentiate into memory B cells or rapidly change their gene expression program and develop into germinal center (GC) and plasma cells (PC). During GC formation pre GC B cells upregulate the expression of the transcriptional repressor Bcl6 that controls the GC B cell differentiation. Bcl6-deficiency results in a complete block of GC B cell reaction, necessary for the generation of high-affinity antibodies by somatic hypermutation and class-switch recombination. In contrast, plasma cell generation occurs normally in Bcl6-deficient mice (Dent et al., 1997; Fukuda et al., 1997). The transcription factor IRF8 directly regulates, possibly in concert with other transcription factors, Bcl6 upregulation in GC B cells (Lee, C. H. et al., 2006). Bcl6 is able to repress several targets including the transcriptional repressor Blimp1 (B lymphocyte induced maturation protein 1) (Shaffer et al., 2000; Tunyaplin et al.,



2004). Therefore, during the GC reaction, Bcl6 represses the gene program for plasma cell generation in GC B cells (Shaffer et al., 2000).

Blimp1 is a key transcription factor for plasma cell (PC) differentiation, where it initiates a gene program, which leads to the inhibition of cell division, to the repression of genes defining the identity of GC B cells, and to the induction of genes necessary for Ig secretion (Kallies et al., 2007; Shaffer et al., 2002). Besides Blimp1, the transcription factors XBP-1 and IRF4 play an essential role for PC differentiation (Sciammas et al., 2006; Shaffer et al., 2004). During PC generation the GC gene program should be downregulated, which is achieved by Blimp1 that represses the expression of Bcl6 and also Pax5 (Diehl et al., 2008; Lin, K. I. et al., 2002) (Figure 5).

In general, the hierarchical expression and the cooperative action of transcription factors as well as epigenetic modulators cause the initiation of a gene program characteristic and irreversible for a certain committed lineage. However, under certain conditions, committed lineages exhibit a high degree of plasticity. For example, TLR engagement drives lymphoid progenitor cells to differentiate into dendritic cells (Nagai et al., 2006), a mechanism that possibly ensures the generation of sufficient numbers of myeloid cells during an acute infection. Today we know that the overexpression of few transcription factors Oct3/4, Sox2, c-Myc and Klf4 in adult murine or human fibroblasts can re-differentiate these cells into multipotent embryonic stem cell-like cells with pluripotent potential *in vitro* as well as *in vivo* (Takahashi et al., 2007; Takahashi & Yamanaka, 2006; Wernig et al., 2007). Therefore, it is not longer surprising that in the hematopoietic system the overexpression of lineage-determining transcription factors in committed cells leads to re-differentiation and lineage conversion. Thus, T cell progenitors could be converted into dendritic cells and mast cells by ectopic expression of PU.1 or GATA-3, respectively (Laiosa et al., 2006; Taghon et al., 2007). Also B cells could be re-differentiated into macrophages upon overexpression of C/EBP $\alpha$  (Xie et al., 2004). Nevertheless, these studies revealed the high instructive capacity of lineage-determining transcription factors.

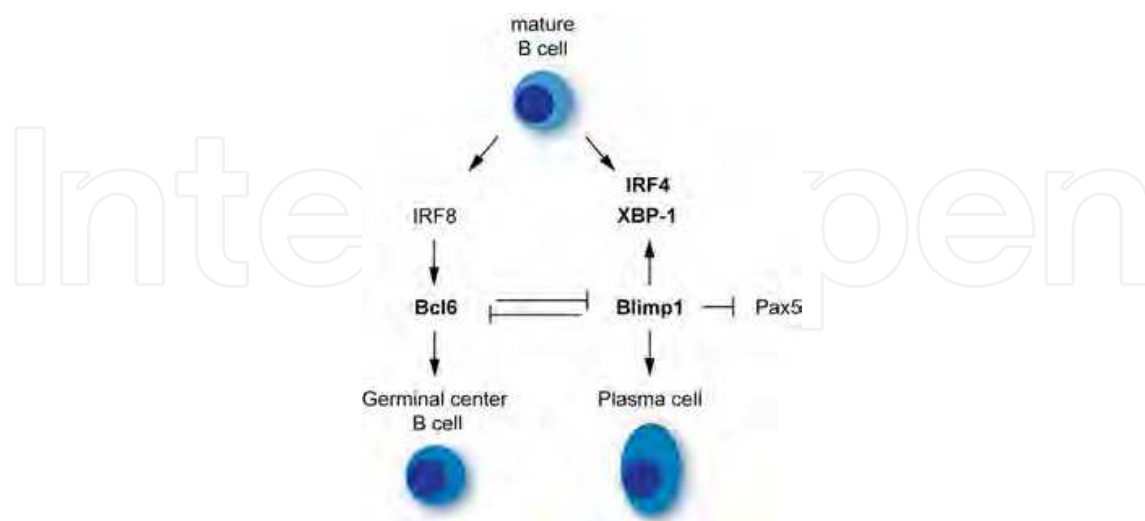


Fig. 5. Cross-regulatory control of germinal center B cell versus plasma cell fate.

Cell fate decision of mature B cells upon antigen exposure is regulated by key transcription factors (bold) that activate cell-specific genes and mutually repress transcription factors necessary for alternative cell differentiation.

In B cells, the lineage commitment and the maintenance of the B cell fate throughout B cell development is achieved by a single transcription factor – Pax5 (Cobaleda et al., 2007; Nutt, S. L. et al., 1999). As already mentioned, deletion of Pax5 leads to a block of B cell development at the pro B cell stage and Pax5<sup>-/-</sup> pro B cells can be re-differentiate in the presence of appropriate cytokines into osteoclasts, NK cells, dendritic cells, macrophages and granulocytes (Nutt, S. L. et al., 1999). More recently it was shown, that the conditional deletion of Pax5 in mature B cells from peripheral lymphoid organs, despite their advanced differentiation state, leads to a de-differentiation back to early uncommitted progenitors in the bone marrow, which even rescued T cell development in T cell-deficient mice (Cobaleda et al., 2007). However, the molecular mechanisms for these reprogramming processes are not finally clear. Since the complete loss of Pax5 in mature B cells also caused the development of aggressive lymphomas, Pax5 was identified as a tumor suppressor for the B cell lineage (Cobaleda et al., 2007).

#### 1.4.2.4 T cell lineage commitment

Multiple bone marrow-derived hematopoietic precursor populations that belong mainly to the MPP or the CLP subsets are able to enter the thymus (Saran et al., 2010; Serwold et al., 2009), where they represent the population of early thymic progenitors (ETP), the initial source for the development of T cells. At this developmental stage the ETP still retain beside the T cell developmental potential also the capability to develop into B cells, macrophages, granulocytes, dendritic cells, and NK cells. Thymic environmental factors, like IL-7, Kit-ligand as well as ligands activating Notch signaling, operate in an inductive manner to force T cell development (Petrie & Zuniga-Pflucker, 2007) and at the same time to down-modulate the capacity to develop into the NK, B or myeloid lineage. Notch signaling blocks these alternative developmental processes and, in addition, is necessary to maintain T cell specification and differentiation (Feyerabend et al., 2009; Franco et al., 2006; Laiosa et al., 2006; Schmitt et al., 2004; Taghon et al., 2007). Very recently it became evident, that besides blocking alternative lineage development Notch signaling drives T cell lineage commitment by upregulating the expression of T lineage-specific transcription factors like TCF-1 necessary for the induction of several T cell-specific genes, like *GATA-3*, *Bcl11b*, and genes coding for components of the T cell receptor (Weber et al., 2011). The Krueppel-like C2H2 type zinc finger transcription factor *Bcl11b* in turn is required for the repression of NK cell associated genes as well as for the downregulation of stem cell or progenitor cell genes not longer required for committed T cells (Li, L. et al., 2010) (Figure 6).

After initial T lineage commitment a subsequent lineage decision is made – the choice to develop into either  $\alpha\beta$  or  $\gamma\delta$  T cell sub-lineages. At the double negative stage (DN; CD4-CD8) thymocytes begin to rearrange their TCR $\beta$ ,  $\gamma$  and  $\delta$  genes. These cells that productively rearranged their TCR $\gamma$  and  $\delta$  genes develop to  $\gamma\delta$  T cells, which remain largely CD4-CD8-. Thymocytes that rearranged efficiently their TCR $\beta$  locus are committed to the  $\alpha\beta$  lineage and express a pre-TCR complex composed of functional TCR $\beta$  chains paired with the invariant pre-TCR $\alpha$  (pT $\alpha$ ) chain. Committed  $\alpha\beta$  T cells undergo a strong proliferative burst and develop further to CD4<sup>+</sup>CD8<sup>+</sup> double positive (DP) thymocytes that start to rearrange their TCR $\alpha$  locus. The precise mechanisms by which DN thymocytes develop into  $\alpha\beta$  or  $\gamma\delta$  T cells are not well understood. Currently mainly two models are discussed: the stochastic and the TCR signal strength model, where strong TCR signals favor  $\gamma\delta$  and weak signals  $\alpha\beta$  lineage choice (reviewed in (Kreslavsky et al., 2010)). Beside TCR signaling also the

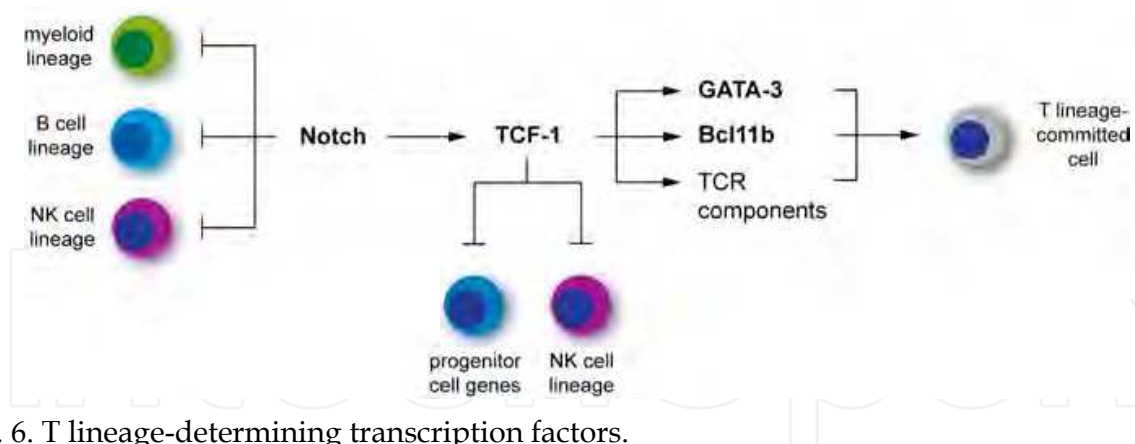


Fig. 6. T lineage-determining transcription factors.

The key transcription factor for T lineage commitment is Notch that suppresses lineage-foreign gene programs and upregulates the lineage-determining transcription factor TCF-1. Finally, lineage commitment is achieved by upregulation of the transcription factors GATA-3 and Bcl11b. Transcription factors are highlighted in bold.

Lymphotoxin-mediated as well as Notch signaling are important for the  $\alpha\beta$  versus  $\gamma\delta$  lineage commitment (Ciofani et al., 2004; Garbe et al., 2006; Garcia-Peydro et al., 2003; Hayes et al., 2005; Kang et al., 2001; Silva-Santos et al., 2005; Van de Walle et al., 2009). Additionally, several transcription factors were identified as important regulators of  $\alpha\beta$  versus  $\gamma\delta$  lineage decision. The high-mobility group transcription factor Sox13, for example, promotes  $\gamma\delta$  T cell development while opposing  $\alpha\beta$  T cell differentiation by antagonizing TCF-1 (Melichar et al., 2007), which is required, similar to ROR $\gamma$ t (Guo, J. et al., 2002), for the survival of CD4<sup>+</sup>CD8<sup>+</sup>  $\alpha\beta$  thymocytes (Ioannidis et al., 2001). Also, the TCR-signal strength dependent upregulation of the Zn-finger transcription factor ThPOK (T-helper inducing POZ-Krueppel factor) was shown to be an important regulator of  $\gamma\delta$  T cell development and maturation (Park, K. et al., 2010). Additionally, by integrating TCR and Notch signals as well as by interacting with and thereby suppressing E protein targets, also the helix-loop-helix transcription factor Id3 promotes  $\gamma\delta$  T cell fate (Lauritsen et al., 2009). The AP-1 family member c-Jun in turn controls directly the expression of the *IL-7R $\alpha$*  gene important for thymocyte development. Deletion of c-Jun results in an enhanced  $\gamma\delta$  T cell generation indicating the importance of IL-7 receptor signaling for the regulation of  $\alpha\beta/\gamma\delta$  T cell fate decision (Riera-Sans & Behrens, 2007).

CD4<sup>+</sup>CD8<sup>+</sup> DP cells expressing a mature  $\alpha\beta$ TCR further undergo positive and negative selection processes based on their ability to recognize self-peptide:self-MHC-complexes as well as their affinity to such complexes. During these selection processes DP cells develop to functionally competent single positive CD4<sup>+</sup>CD8<sup>-</sup> or CD4<sup>+</sup>CD8<sup>+</sup> T cells equipped with a specific gene expression program characteristic for CD4<sup>+</sup> T helper or CD8<sup>+</sup> cytotoxic T lymphocytes. Mainly two transcription factors – ThPOK and Runx3 – are important for directing the development of DP thymocytes either into the CD4<sup>+</sup> T helper or CD8<sup>+</sup> cytotoxic T cell population (Egawa & Littman, 2008; He et al., 2008; Taniuchi et al., 2002; Wang, L. et al., 2008). Therefore, ThPOK is required for the commitment to CD4<sup>+</sup> T helper cells by repressing the characteristic genes for CD8<sup>+</sup> cells including Runx3, whereas Runx3 mediates the silencing of the *CD4* locus in CD8<sup>+</sup> cells. These dual regulative processes, leading to the exclusion of Runx3 expression in CD4<sup>+</sup> cells by ThPOK as well as the exclusion of the expression of ThPOK in CD8<sup>+</sup> cells by Runx3, result finally in CD4-CD8 lineage

commitment. Transcription factors involved in ThPOK upregulation in MHCII-restricted T lymphocytes are GATA-3 (Wang, L. et al., 2008) together with the HMG protein Tox (Aliahmad & Kaye, 2008). In contrast, IL-7-mediated activation of the STAT5 transcription factor promotes the upregulation of Runx3 in CD8<sup>+</sup> cells (Park, J. H. et al., 2010) indicating a differential requirement of cytokine signaling for CD4 and CD8 lineage development. After CD4<sup>+</sup> or CD8<sup>+</sup> single positive  $\alpha\beta$  T cells are generated they are ready to leave the thymus and enter via the blood stream peripheral lymphatic organs, where their terminal differentiation occurs.

From the CD4<sup>+</sup>CD8<sup>+</sup> DP pool of thymocytes not only conventional  $\alpha\beta$  T cells arise, but also natural killer (NK) T cells. In contrast to conventional  $\alpha\beta$  T cells that are restricted by MHCI or MHCII molecules, invariant NK T cells undergo positive and negative selection processes during their thymic maturation, which are mediated by the recognition of glycolipids presented by the MHCI-like molecule CD1d. Additionally, they also require signals from the Slam family of receptors. Different types of NK T cells are described. However, the most common and best-studied NK T cells are the invariant NK (iNK) T cells expressing an invariant TCR that is composed of a common  $\alpha$ -chain in combination with a certain number of  $\beta$ -chains. After antigen recognition iNK T cells secrete high amounts of a large variety of cytokines and chemokines within minutes. Therefore, these cells exhibit rather an innate than an adaptive immune function. Several transcription factors were identified to be important for iNK T cell lineage choice. Among them, the transcription factor PLZF (promyelocytic leukemia zinc finger) is a key regulator for the development of this particular cell type (Kovalovsky et al., 2008; Savage et al., 2008), since in the thymus it is exclusively expressed by iNK T cells. In addition, several other transcription factors like NF- $\kappa$ B (Sivakumar et al., 2003; Stanic et al., 2004), Ets-1 (Lacorazza et al., 2002; Walunas et al., 2000), GATA-3 (Kim, P. J. et al., 2006), T-bet (Matsuda et al., 2006) and Runx proteins (Egawa et al., 2007) contribute to the development, differentiation and survival of iNK T cells. Because these transcription factors are also expressed in other thymic subpopulations they are not exclusively important for the iNK T cell lineage. However, PLZF-deficiency did not prevent iNK T cell development in general but severely interfered with iNK T cell effector differentiation and therefore with their functionality (Kovalovsky et al., 2008; Savage et al., 2008).

In the thymus, a subpopulation of MHC-II-restricted CD4<sup>+</sup> T cells further differentiates into CD25<sup>+</sup> naturally occurring regulatory T cells (nTregs) characterized by the expression of the transcription factor FoxP3 (Fontenot et al., 2003; Hori et al., 2003). They comprise about 5 to 10% of peripheral CD4<sup>+</sup> T cell and play a crucial role for maintaining peripheral tolerance. nTregs are able to suppress the proliferation, cytokine secretion as well as activation of autoreactive effector T cells thereby preventing autoimmunity. FoxP3 plays an essential function for the regulation of nTregs suppressive activity, since the deficiency of a functional FoxP3 leads to a severe autoimmune pathology in mouse (Godfrey et al., 1991; Lyon et al., 1990) and man (Bennett et al., 2001; Wildin et al., 2001). Several transcription factors are implicated in the *FoxP3* gene regulation and therefore for the development and function of nTregs. After activation of PKC $\theta$  and/or CD28 engagement, Notch3 together with NF- $\kappa$ B heterodimers composed of p50/p65 are able to bind and to trans-activate the FoxP3 promoter *in vivo* (Barbarulo et al., 2011; Soligo et al., 2011). Also NF- $\kappa$ B c-Rel was identified as a factor able to initiate FoxP3 transcription in thymic Treg precursors (Deenick et al., 2010; Isomura et al., 2009; Long et al., 2009; Ruan et al., 2009). Additionally, the FoxP3 promoter



contains several functional NFAT/AP-1 binding sites, which are occupied *in vivo* (Mantel et al., 2006). Moreover, the transcription factor Bcl11b is also able to promote directly FoxP3 as well as IL-10 expression. Deletion of Bcl11b at the DP stage of thymic T cell development or solely in Tregs causes inflammatory bowel disease – a severe autoimmune disorder – due to reduced Treg suppressor activity accompanied with reduced FoxP3 and IL-10 expression (Vanvalkenburgh et al., 2011).

Conventional  $\alpha\beta$  T cells leave the thymus and settle peripheral lymphatic organs as naïve T cells. After activation by exposure to their cognate antigens naïve CD4<sup>+</sup> cells differentiate into an appropriate T helper cell (TH) lineage that plays an essential role in acquired immunity. Depending on the cytokine milieu produced by antigen presenting cells naïve CD4<sup>+</sup> cells undergo differentiation processes resulting in the expression of master transcription factors defining the ability to secrete a certain set of cytokines. Initially, two main TH subpopulations were described (Mosmann et al., 1986). The generation of TH1 cells depends on the presence of IFN $\gamma$  and/or IL-12 inducing the expression of the master transcription factor T-bet essential for the TH1 phenotype characterized by the production of large amounts of IFN $\gamma$ , IL-2 and TNF $\alpha$ . TH1 cells mediate the defense against infections by intracellular microbes and the isotype switching to IgG2a and IgG2b. In contrast, TH2 cells are generated in the presence of IL-4 and also secrete, depending on the upregulation of the transcription factor GATA-3, IL-4 together with IL-5 and IL-13. Thereby, humoral responses against parasites and extracellular pathogens are supported and also the class switching to IgG1 and IgE (Mosmann et al., 1986; Mowen & Glimcher, 2004; Szabo et al., 2003). A third TH subpopulation was described, the TH17 cells that is characterized by the secretion mainly of IL-17A and IL17F, but also IL-21 and IL-22, protecting the host against bacterial and fungal infections. Their differentiation is induced by TGF $\beta$  together with IL-6 or IL-21, which prompts the expression of the master transcription factor essential for TH17-development – ROR $\gamma$ t (Ivanov et al., 2006). More recently, two additional TH subsets were described – TH9 and TH22 expressing predominantly the cytokines IL-9 or IL-22, respectively. The development of TH9 cells is initiated upon antigen receptor stimulation in the presence of IL-4 and TGF $\beta$  (Dardalhon et al., 2008; Veldhoen et al., 2008) and requires the upregulation of the transcription factor PU.1 (Chang et al., 2010). TH22, identified in the human skin, are characterized by the expression of the chemokine receptors CCR6, CCR4 and CCR10 as well as by the transcription factor aryl hydrocarbon receptor (AHR) that might be involved in the regulation of IL-22 gene expression (Duhon et al., 2009; Trifari et al., 2009).

Another T helper subtype that differentiates in the periphery from naïve CD4<sup>+</sup> cells is the follicular T helper (TFH) cell subpopulation, characterized by the expression of CXCR5, ICOS and PD-1 as surface markers. They synthesize large quantities of IL-21 and require the upregulation of the transcriptional repressor Bcl6 for their development and also for their function to promote germinal center B cell maturation (Johnston et al., 2009; Nurieva et al., 2009; Yu et al., 2009). Bcl6 expression is regulated by IL-6 and IL-21 (Nurieva et al., 2009) and drives not only the TFH differentiation but also inhibits the development of other CD4<sup>+</sup> differentiation pathways by blocking Blimp1 (Johnston et al., 2009).

In the periphery, CD4<sup>+</sup> effector T cells can be converted by exposure to TGF $\beta$  and IL-2 to inducible regulatory T cells (iTregs) expressing CD25 at the surface and, like nTregs, FoxP3 as a master transcription factor necessary for Treg function (Davidson et al., 2007; Zheng et al., 2007) (Figure 7).



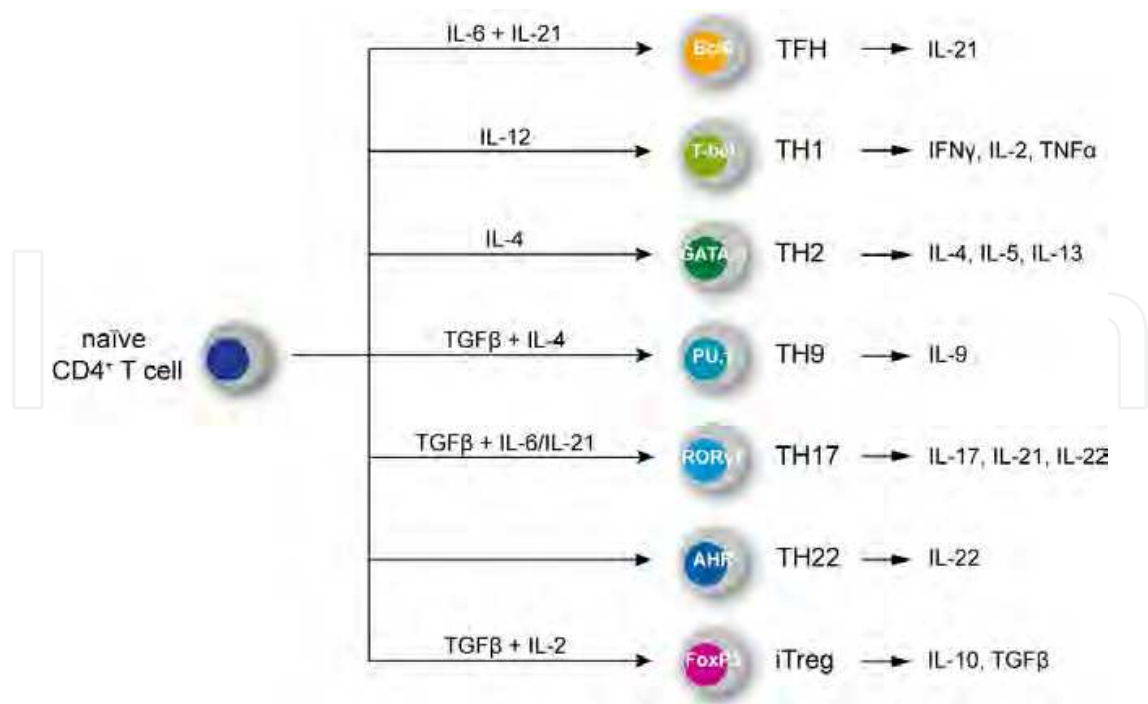


Fig. 7. Terminal Differentiation of CD4<sup>+</sup> T cells.  
Differentiation of CD4<sup>+</sup> T cells into different T helper cell subpopulations after antigen exposure is driven by the specific cytokine milieu and results in the expression of specific transcription factors (noted in white). Every T helper cell subset releases distinct cytokines, which modulate the immune response of the host.

CD4<sup>+</sup> T cell master transcription factors as well as lineage-specific cytokines characteristic for the appropriate TH subpopulations are able to block the differentiation of other TH subsets. For example, T-bet in cooperation with Runx3 suppresses the generation of TH2 cells by physical interaction with GATA-3 thereby inhibiting GATA-3 activity (Djuretic et al., 2007; Hwang et al., 2005). Additionally, T-bet also actively represses TH17 differentiation by preventing Runx1-mediated upregulation of ROR $\gamma$ t expression (Lazarevic et al., 2011). Also, as already noted, Bcl6 expressed by TFH antagonizes Blimp1 and thereby it inhibits the developmental program necessary for alternative TH cell differentiation (Johnston et al., 2009). However, several studies suggest certain plasticity in the expression of master transcription factors as well in the set of cytokines that differentiated T helper cells secrete. The conversion of peripheral effector CD4<sup>+</sup> cells to iTregs expressing FoxP3 like nTregs that developed in the thymus was a first hint indicating plasticity of CD4<sup>+</sup> TH cells (Jonuleit et al., 2001). Moreover, in the presence of TGF $\beta$  TH2 cells can acquire IL-9 producing capacity (Veldhoen et al., 2008). Additionally, several studies described the acquisition of IFN $\gamma$ -producing potential by TH17 cells *in vivo* in mouse and man (Kurschus et al., 2010; Wilson, N. J. et al., 2007) and even a complete conversion of TH17 cells into IFN $\gamma$ -producers (Bending et al., 2009; Lee, Y. K. et al., 2009; Shi et al., 2008). When stimulated with IL-4, TH17 cells can change into IL4-secreting TH2 cells (Yi et al., 2009). Also, Tregs stimulated with IL-6 can express IL-17 and downregulate FoxP3 expression (Xu et al., 2007). Together, these data indicate the high flexibility of peripheral CD4<sup>+</sup> cells in their potential to secrete a certain set of cytokines and therefore to modulate and/or influence the outcome of an ongoing immune response. However, the mechanism(s) underlying the plasticity of “committed” TH cells remain largely unclear.

### 1.4.3 Epigenetic mechanisms controlling hematopoiesis

The highly coordinated program needed to pass through the diverse developmental stages that comprise hematopoiesis can only be achieved by tight regulation. In fact, every cellular transition and differentiation step is characterized by the activation of a new, lineage-specific, genetic program and the extinction of the previous one. This is achieved by the action of well-defined networks of transcription factors at each developmental step as already described. However, transcription factors are not the only players in the complex differentiation process of hematopoiesis, since there is an increasing body of evidence demonstrating that the regulation of hematopoietic stemness and lineage commitment is dependent on epigenetic mechanisms. Chromatin, the higher order structure of DNA and nucleosomes, can adopt different structural conformations depending on epigenetic modifications, which influence the accessibility of DNA for the gene transcription machinery. Four types of epigenetic regulation can take place: DNA methylation, histone modification, chromatin remodeling and gene silencing via microRNAs.

#### 1.4.3.1 DNA methylation

DNA methylation of cytosines at CpG dinucleotides, except for CpG islands, is established during early embryogenesis by DNA methyltransferases (Dnmt) and is maintained in somatic cells to repress transcription. The DNA methyltransferases Dnmt3a and Dnmt3b are supposed to convey *de novo* methylation, whereas Dnmt1 conserves previously installed methylation states during replication. First hints depicting the importance of DNA methylation for hematopoietic development arose from gene deletion studies in mice revealing the indispensable functions of Dnmt1 for HSC self-renewal and lineage-commitment. The ablation or reduced expression of Dnmt1 in murine HSC led to diminished repopulating capacity of HSC and decreased production of lymphoid progenitors accompanied with retained myelo-erythroid progenitor development (Broske et al., 2009; Trowbridge et al., 2009). Additionally, the examination of genome-wide methylation profiles of the mouse hematopoietic system demonstrated methylation pattern changes during differentiation resulting in the activation of silent genes and the silencing of active genes. Moreover, the study could show that myeloid commitment involved less global DNA methylation than lymphoid commitment (Ji et al., 2010) in line with the findings from the Dnmt1 deletion studies. In contrast to Dnmt1, Dnmt3a/3b deficiency affected only the long-term reconstitution ability of HSC, but not their differentiation into committed progenitors (Tadokoro et al., 2007). Nevertheless, the molecular mechanisms mediating DNA methylation and demethylation during hematopoietic development have not been deciphered, although chromatin-remodeling factors as well as Polycomb group/Dnmt3a/3b complexes recruited by transcription factors were supposed to be involved (Gao et al., 2009; Kirillov et al., 1996; Vire et al., 2006).

#### 1.4.3.2 Histone modification

Another crucial epigenetic mechanism is the posttranslational modification of histones, which embraces acetylation, methylation, phosphorylation and sumoylation among others. These modifications occur at the tails of histones and change the direct interactions between nucleosomes and DNA, thereby affecting gene expression (Campos & Reinberg, 2009). In terms of hematopoietic regulation the methylation of lysine 4 (K4) and 27 (K27) of histone 3 (H3) particularly have to be stressed, since they can serve as repressing, activating and

poising marks dependent on the methylation pattern. The concomitant trimethylation of H3K27 (repressing mark) and H3K4 (activating mark) as well as the mono- and dimethylation of H3K4 introduce a bivalent epigenetic modification leading to poised chromatin that is primed for activation of gene transcription (Bernstein, B. E. et al., 2006; Heintzman et al., 2009; Orford et al., 2008). Several studies have demonstrated that a plethora of lineage-specific genes are poised at the beginning of hematopoiesis or achieve poising marks during hematopoietic differentiation. After commitment of the cell to a specific lineage, lineage-foreign genes lose their poising marks and repression of gene transcription occurs (Orford et al., 2008; Weishaupt et al., 2010). Moreover, genome-wide analysis of poised chromatin sites revealed a tight correlation of bivalent histone methylation sites with binding sites of lineage-determining transcription factors such as EBF1, E2A, GATA-1 or PU.1. These sites are independent of the transcription start site and are probably enhancer sites that are involved in the priming for transcriptional activation in later stages of hematopoietic development (Heintzman et al., 2009; Heinz et al., 2010; Lin, Y. C. et al., 2010; Treiber et al., 2010). Similar to DNA methylation, the molecular mechanisms underlying histone modifications have not yet been identified.

#### 1.4.3.3 Chromatin remodeling

Additional epigenetic modifiers of DNA accessibility that were recruited through lineage-specific transcription factors are chromatin-remodeling complexes. Such chromatin remodelers are multi-protein complexes that are able to change nucleosome location or conformation in an ATP-dependent manner, but they additionally contain interchangeable histone modifying enzymes such as deacetylase or acetylase to produce functionally distinct complexes (Bowen et al., 2004). For example, Ikaros, a lymphoid-specific transcription factor crucial for the commitment of LMPP into CLP can recruit Mi2/NuRD complexes in order to repress genes (Kim, J. et al., 1999; Koipally et al., 1999; Sridharan & Smale, 2007). Whereas, EBF1 and E2A are involved in the recruitment of the SWI/SNF complex to the upstream enhancer of the *CD19* locus as well as to the *CD79a* promoter region facilitating the transcriptional activation of these B cell-specific genes (Gao et al., 2009; Walter et al., 2008).

#### 1.4.3.4 MicroRNAs

Besides the already mentioned epigenetic mechanisms established at the level of DNA, the recent discovery of microRNAs (miRNAs) added a further layer of epigenetic regulation that guides the hematopoietic differentiation process. These mRNAs are small, single-stranded, non-coding RNAs, which are able to repress mRNA transcription by the promotion of mRNA degradation due to direct binding to the 3' untranslated regions (UTR) of specific target mRNAs. The first evidences for the importance of miRNAs during hematopoietic development revealed from the deletion of Dicer, an RNase-III-like enzyme that is indispensable for miRNA biogenesis, in mice. These gene ablation leads to embryonal lethality at day 7.5 due to a lack of detectable multipotent stem cells, whereas the conditional deletion in murine embryonic stem cells blocks the ability to differentiate (Bernstein, E. et al., 2003; Kanellopoulou et al., 2005) and the lineage-specific ablation of Dicer in lymphoid progenitors results in severe defects in the B as well as T cell development (Cobb et al., 2005; Koralov et al., 2008; Muljo et al., 2005). Moreover, analyses of miRNA expression in several subsets of human CD34<sup>+</sup> HSC and progenitors cells as well as murine hematopoietic tissues have demonstrated the modulated transcription of different miRNA during hematopoiesis (Chen et al., 2004; Georgantas et al., 2007; Liao et al., 2008). With

regard to the relative young field of miRNA research, only limited data about the detailed role of single miRNAs in the different steps of HSC maintenance or hematopoietic differentiation are available, but some regulatory mechanisms are already described. At the level of HSC, where decision-making comprises self-renewal and differentiation into committed progenitors, miRNAs of the miR-196 and miR-10 family are highly expressed, which are able to modulate HSC homeostasis and lineage commitment through the regulation of certain *HOX* genes (Mansfield et al., 2004; Yekta et al., 2004), whereas miR-125a has been shown to mediate self-renewal of LT-HSC by targeting the pro-apoptotic protein *Bak-1* (Guo, S. et al., 2010). In contrast, miR-126 conferring lineage commitment and progenitor production via down-modulation of *HOXA9* and the tumor suppressor polo-like kinase 2 that has to be downregulated during differentiation towards multipotent progenitors (Shen et al., 2008).

Downstream of HSC, the introduction of lineage commitment is the most important task of a regulatory mechanism and several miRNAs are involved in these processes in the different progenitor populations. For example, during erythroid lineage differentiation starting from the MEP a progressive downregulation of miR-24, miR-221, miR-222 and miR-223 as well as a upregulation of miR-451 and miR-16 has been reported in differentiating human erythroid progenitors (Bruchova et al., 2007). The down-modulation of miR-221 and miR-222 is necessary for the expression of Kit that in turn allows the expansion of erythroblasts (Felli et al., 2005), whereas the repression of miR-24 permit the expression of activin type I receptor, which promotes erythropoiesis in cooperation with erythropoietin (Wang, Q. et al., 2008). A further activator of erythroid differentiation is the transcription factor and miR-223-target LIM-only protein 2 that along with GATA-1 and others constitutes a multi-protein complex (Felli et al., 2009). In contrast to these down-modulated miRNAs, miR-451 upregulation is indispensable for erythroid maturation and effective erythropoiesis in response to oxidative stress. Several studies have demonstrated that miR-451 targets 14-3-3 $\zeta$ , a chaperone protein modulating intracellular growth factor signals, and therefore regulating the expression of several genes associated with late erythropoiesis (Patrick et al., 2010; Rasmussen et al., 2010; Zhan et al., 2007). But also megakaryocyte differentiation occurs downstream of the MEP and several studies revealed the importance of miR-150 for lineage commitment during megakaryocyte-erythroid differentiation, since the ectopic expression of miR-150 in MEP drives the differentiation towards megakaryocytes at the expense of erythroid cells by targeting the transcription factor *c-Myb* (Lu et al., 2008). Further support for the lineage-determining function of miR-150 has arose from a study demonstrating the regulation of miR-150 and *c-Myb* through the megakaryocyte-specific cytokine TPO (Barroga et al., 2008). Other miRNAs downregulated during megakaryopoiesis are miR-130 targeting *MafB* that in turn together with GATA-1 is needed for the induction of the *Gbilb* gene (Garzon et al., 2006) as well as miR-155 that targets the transcription factors *Ets-1* and *Meis-1* (Romania et al., 2008).

With respect to the role of miRNAs in myelopoiesis miR-223 has to be enumerated, which also functions as lineage-determining factor that is upregulated during granulopoiesis and downregulated during monopoiesis (Fazi et al., 2005; Johnnidis et al., 2008). Targets of miR-223 are the cell cycle regulator *E2F1* and the monocyte lineage-promoting gene *Mef2c* leading to suppression of proliferation and induction of granulocyte differentiation (Johnnidis et al., 2008). Moreover, the transcription of miR-223 is activated by the master transcription factor for granulopoiesis C/EBP $\alpha$  that replaces the transcriptional repressor NFI-A upon activation of granulocytic differentiation (Fazi et al., 2005). A similar mechanism has been



recently described for miR-34a that is also increased expressed during granulopoiesis by C/EBP $\alpha$ -mediated transcription and targets the cell cycle regulator *E2F3* (Pulikkan et al., 2010). Another lineage-determining mechanism displays the repression of miR-21 and miR-196b by the transcriptional repressor Gfi1 during granulopoiesis, since ectopic expression of both miRNAs in myeloid progenitors results in a complete block of G-CSF induced granulopoiesis (Velu et al., 2009). In favor of monocytic development acts the activation of miR-424 transcription via PU.1 that in turn targets the negative regulator of monopoiesis *NFI-A* (Forrest et al., 2010). Whereas the miR-17/miR-20/miR-106 cluster is repressed during monopoiesis in humans, probably to allow expression of the target AML-1 that consecutively promotes monocyte-macrophage differentiation and maturation (Fontana et al., 2007).

Concerning the function of single miRNAs during lymphopoiesis only few data are available, despite the astonishing effects of Dicer deletion on lymphoid development. However, one study partially explains the phenotype of Dicer deletion by the defective expression of the miR17-92 cluster. This miRNA cluster that is highly expressed in progenitor cells targets the pro-apoptotic factors *Bim* and *Pten*. In line with these findings,

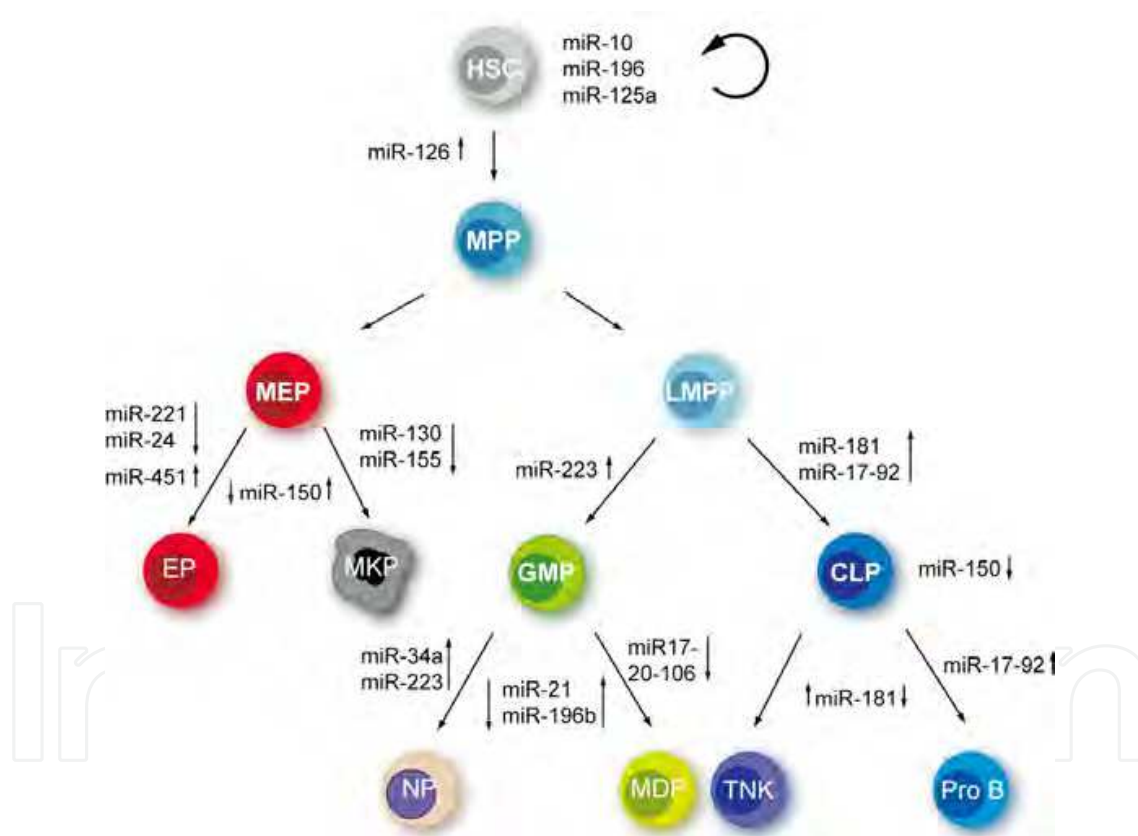


Fig. 8. The regulatory network of miRNAs during hematopoiesis.

Several miRNAs are involved in maintenance of HSC self-renewal, whereas other miRNAs are associated with lineage commitment and development towards differentiated progeny. HSC, hematopoietic stem cell; MPP, multipotent progenitor; LMPP, lymphoid-primed multipotent progenitor; CLP, common lymphoid progenitor; CMP, common myeloid progenitor; MEP, megakaryocyte-erythrocyte progenitor; GMP, granulocyte-macrophage progenitor; NP, neutrophil progenitor; MDP, monocyte-dendritic cell progenitor; TNK, T cell NK cell progenitor; EP, erythroid progenitor; MKP, megakaryocyte progenitor.



ablation of the miR17-92 cluster in mice results in a severe block of B cell development at the pro B to pre B transition due to increased apoptosis of pro B cells (Ventura et al., 2008). A similar block in B cell development at the pro B to pre B transition is caused by the ectopic expression of miR-150 in lymphoid progenitors due to the repression of the transcriptional repressor *c-Myb* (Xiao et al., 2007). The best-described miRNA involved in T lymphocyte development is miR-181, which promotes T cell differentiation through increasing signaling strength of the TCR signaling. In detail, miR-181 targets multiple phosphatases such as *PTPN22* or *DUSP5* and *DUSP6*, which are negative regulators of distinct steps of the TCR signaling pathway leading to an upregulation of ERK1/2 phosphorylation upon TCR engagement. This increased sensitivity of TCR signaling is needed during the positive selection of double-positive T cells in the thymus (Li, Q. J. et al., 2007) (Figure 8).

#### 1.4.4 Role of cytokines in guiding hematopoiesis

Cytokines are a large family of extracellular ligands that stimulate several responses after binding to structurally and functionally conserved cytokine receptors. Biological responses provoked by cytokines cover a broad spectrum of different biological activities, for example survival, proliferation, differentiation, or maturation. In the case of the hematopoietic system, the most important cytokines are interleukins and colony-stimulating factors with supportive functions for several lineages as well as erythropoietin (EPO) and thrombopoietin (TPO) that act on single lineages (Metcalf, 2008). Besides the requirement of cytokines for regulation of basal hematopoiesis, they are also essential for controlling emergency hematopoiesis in response to infections or blood loss. This is reflected by the different origins of cytokines, secreted for example by activated immune cells or by stroma cells as well as by organs, like liver and kidney.

In steady-state conditions, serum concentrations of cytokines are low, but they can be elevated up to 1000-fold by challenging the immune system and possess high picomolar affinities for their corresponding receptors (Metcalf, 2008). On the binding of cytokine molecules follows the activation of the receptor via homodimerization (G-CSFR), oligomerization with a common signaling subunit (GM-CSFR, IL-6R) or conformational changes in preformed receptor dimers (EPOR), which finally leads to activation of Janus kinases (JAK). Upon activation of the tyrosine kinases of JAK family, the cytokine receptors as well as the kinases themselves are phosphorylated to generate docking sites for SH2 domain containing proteins. One example is the STAT protein family that promotes transcriptional activation of target genes after phosphorylation by JAK (Robb, 2007; Smithgall et al., 2000). Additionally, other signaling molecules can be recruited to the cytokine receptors, such as Src kinases, protein phosphatases or PI3K, which mediate the activation of numerous signaling pathways like MAPK-ERK, Ras or PI3K (Baker et al., 2007).

To date, basically two hypotheses exist concerning the role of cytokines in hematopoiesis. The instructive model proposes that cytokines transmit specific signals to multipotent progenitors to direct their lineage commitment. In contrast, the permissive or stochastic model suggests that cytokines only provide permissive growth and survival signals to intrinsically determined and lineage-committed progenitors. Supportive data for the permissive as well as the instructive model of cytokine function originated from different studies, where cytokine receptors were ectopically expressed in lineage-committed progenitors. Studies in favor of the permissive model have demonstrated that viral

transduction of fetal liver cells with the M-CSF receptor results in the generation of erythroid colonies upon M-CSF administration (McArthur et al., 1994). Similar results have been obtained by the restoration of definitive erythropoiesis in EPOR-deficient fetal liver cells via the expression of the human GM-CSFR plus GM-CSF treatment (Hisakawa et al., 2001). Furthermore, replacement of the intracellular domain of the G-CSFR with the intracellular domain of EPOR induces no alterations in lineage commitment (Semerad et al., 1999). Oppositional results emanated from the ectopic expression of IL-2R $\beta$  in CLP, which results in rapid generation of granulocytes and macrophages in the presence of IL-2 (Kondo et al., 2000).

Additionally, ectopic expression of the human GM-CSFR in IL-7-deficient CLP was not able to restore lymphopoiesis upon GM-CSF administration (Iwasaki-Arai et al., 2003). Experiments with single GMP cultured in the presence of M-CSF or G-CSF have further supported the hypothesis of lineage instruction by cytokines due to the almost solely development of either macrophages or granulocytes, respectively (Rieger et al., 2009). Nevertheless, gene deletion studies for several cytokine receptors have shown the indispensable function of the most cytokines for hematopoiesis. For example, the knockout of EPO and EPOR in mice leads to embryonic death at E13.5 due to severe anemia, even if erythroid progenitor cells were present (Lin, C. S. et al., 1996; Wu, H. et al., 1995). Analysis of IL-7R $\alpha$ -deficient mice revealed a lethal phenotype as a result of a severe hypoplasia of all lymphoid lineages, but retained development of the earliest unipotent T and B cell precursors (Peschon et al., 1994). In contrast, mice bearing deletions of colony-stimulating factor receptors demonstrated no lethal phenotypes. Disruption of the G-CSFR in mice results in ineffective granulopoiesis, with chronic neutropenia due to a decrease of mature myeloid cells in the bone marrow and a modest reduction of progenitor cells (Liu et al., 1996), whereas deletion of the common  $\beta$ -chain of IL-3, IL-5 and GM-CSF receptor in mice only lead to reduced numbers of eosinophils (Nishinakamura et al., 1996; Nishinakamura et al., 1996).

Taken together, the present available data do not resolve the question if cytokines only have permissive functions in the guidance of hematopoiesis, especially with regard to differentiation from HSC to restricted progenitors or not. But the plasticity of the transition from multipotent progenitor cells to restricted progenitor cells, especially regarding adaption of hematopoiesis in emergency situations suggests that cytokines have to some extent instructive functions. Furthermore, almost nothing is known about potential functions of cytokines and cytokine signaling regarding gene expression or posttranslational regulation of lineage-determining transcription factors, which would provide a possible link between intrinsic and extrinsic regulation of hematopoiesis (Figure 9).

## 1.5 Perspectives

The unique property of the hematopoietic system to replenish permanently all hematopoietic cells together with the ongoing progress in the understanding of hematopoietic differentiation processes allows the development of new therapy approaches to treat hemic diseases such as hematologic malignancies, immunodeficiencies or autoimmune diseases. These new therapies are mainly based on the transplantation of hematopoietic stem cells (HSC). Two basic findings promoted the clinical use of HSC transplantation (HSCT) in the last twenty years. First, the definition of human HSC as

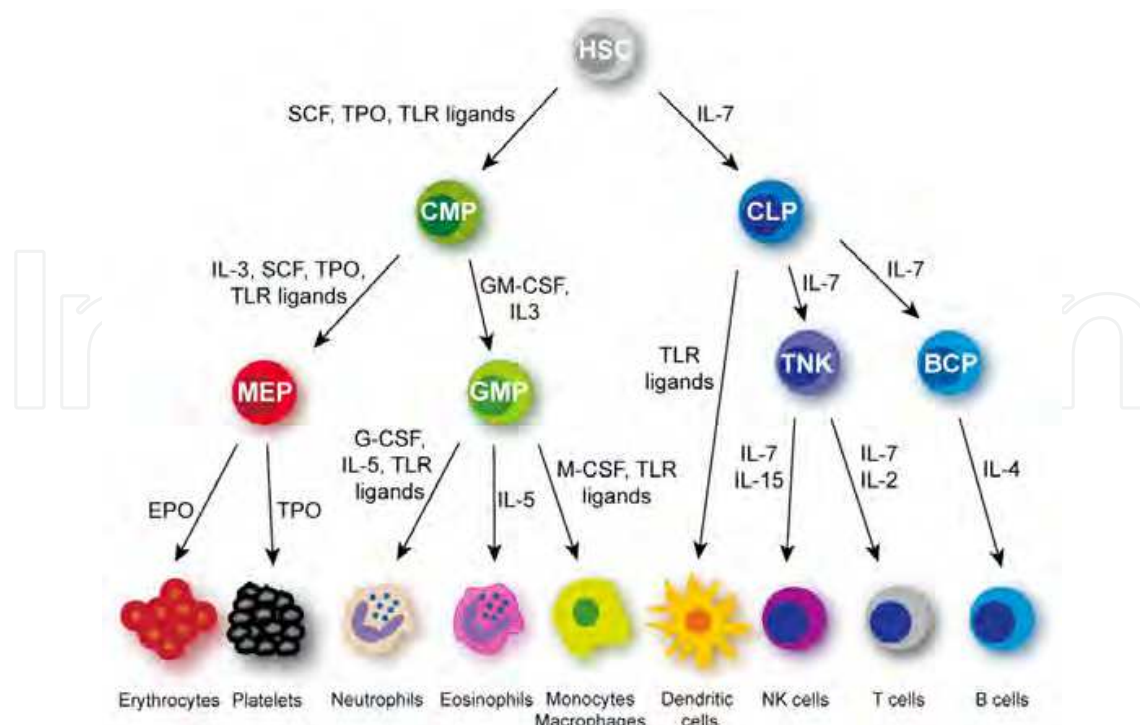


Fig. 9. The role of cytokines in hematopoiesis.

Cytokines act on both multipotent progenitors as well as committed progenitors and provide survival and proliferation signals. HSC, hematopoietic stem cell; CLP, common lymphoid progenitor; CMP, common myeloid progenitor; MEP, megakaryocyte-erythrocyte progenitor; GMP, granulocyte-macrophage progenitor; TNK, T cell NK cell progenitor; BCP, B cell progenitor.

CD34<sup>+</sup>/CD38<sup>-</sup> cells that can be found in the bone marrow as well as in the umbilical cord blood (Weissman & Shizuru, 2008), and second, the discovery of the potent function of the cytokine G-CSF in mobilization of stem cells from the bone marrow into peripheral blood (Weaver et al., 1993). In most cases, this method provides several advantages in comparison to bone marrow transplantation, since the isolation of stem cells from peripheral blood via leukapheresis is less invasive for the donor and the recovery of the hematopoietic system occurs faster (Gertz, 2010).

Currently, only two possibilities of HSCT for the treatment of life-threatening hemic diseases or immunodeficiencies are available: the autologous transplantation or the allogeneic transplantation of HSC from a healthy donor. Autologous transplantation of HSC is often used during the cure of hematologic malignancies like myeloma or some types of lymphoma to restore the hematopoietic system after aggressive chemotherapy (Gertz et al., 2000; Linch et al., 1993; Moreau et al., 2011; Philip et al., 1995). In contrast, allogeneic HSC transplantation (HSCT) is the treatment of choice for many otherwise fatal hematologic malignancies (chronic myeloid leukemia, acute leukemia) and genetic disorders such as aplastic anemia,  $\beta$ -thalassemia as well as primary immunodeficiencies (severe combined immunodeficiency, Wiskott-Aldrich syndrome) and requires an almost perfect human leukocyte antigen (HLA) match of donor and recipient (Roncarolo et al., 2011). Additionally, recent trails of allogeneic HSCT provided good results in the cure of autoimmune diseases like multiple sclerosis and rheumatoid arthritis (Sullivan et al., 2010). Nevertheless, the need

for an almost perfect HLA match to avoid graft-versus-host reactions, the restricted availability of donors as well as pre-transplant conditioning limits the application of allogeneic HSCT today (Roncarolo et al., 2011).

One possible approach to circumvent the potential risks of allogeneic HSCT is the gene therapy, where defective genes are restored by the introduction of functional counterparts in autologous HSC. The first trails of gene therapy for primary immunodeficiencies employed the retroviral transduction of isolated HSC for gene delivery and afterwards the re-transplantation into patients. But in some of this trails, a certain amount of patients developed hematologic malignancies due to random integration of the transgene into the genome that can lead to the trans-activation of proto-oncogenes when the virus sequence is integrated in their vicinity. Recent efforts for the improvement of viral vectors by the use of retro- and lentiviral vectors with cell-specific promoter sequences may provide gene therapy with a decreased incidence for undesired trans-activation of oncogenes. Another approach could be the development of robust methods for homologous recombination in HSC, which would not only avoid the cancer risk, but also allow the endogenous regulation of the corrected gene (Kohn, 2010). Additionally, new perspectives offered the groundbreaking findings of Takahashi and Yamanaka demonstrating the induction of pluripotent stem cells from mouse fibroblasts by the viral transduction of Oct3/4, Sox2, c-Myc and Klf4 (Takahashi & Yamanaka, 2006). Moreover, several recent improvements in induction of pluripotency in several cell types like dermal fibroblasts, keratinocytes and blood cells by the use of virus-free and/or vector-free techniques render reprogrammed somatic cells to a possible future technology for stem cell plus gene therapy (Wu, S. M. & Hochedlinger, 2011). Some of the advantages of iPS (inducible pluripotent stem) cells are the independency of the cell source, the possibility of prolonged culturing *ex vivo* allowing genetic manipulation, and the differentiation into all cell types. A first proof of principle for use of iPS cells in gene therapy supplied the successful treatment of sickle cell anemia in a mouse model. In this study, autologous skin fibroblasts from humanized sickle cell anemia mice were reprogrammed into iPS cells and the genetic defect was repaired via homologous recombination, afterwards the iPS cells were differentiated into hematopoietic progenitors in vitro and finally transplanted into irradiated recipient mice (Hanna et al., 2007). Nevertheless, these types of therapies need a lot of extensive research before they can be applied in the clinic in future, but our increasing knowledge of the regulation of hematopoietic processes provides the basis for the individual treatment of patients with life-threatening hematologic disorders with autologous cells to abolish side effects associated with allogeneic HSCT, or to cure diseases currently not treatable with peripheral stem cell transplantation.

## 2. Conclusion

The process of differentiation and lineage commitment during hematopoietic development depends strongly on the defined activation of lineage-determining gene programs as well as the repression of lineage-foreign gene programs. This concerted regulation of genetic programs can only be achieved by the integration of several mechanisms such as activation of lineage-specific transcription factors, modulation of epigenetic marks and extrinsic signals that provide a supportive environment. Moreover, the described mechanisms of hematopoiesis illustrate the complex network involving the synergistic effects of a certain number of key transcription factors that not only induce transcription but also guide



epigenetic changes to allow or deny the access of the transcriptional machinery to the DNA or the repression of important genes via gene silencing. Additionally, some of these transcription factors are often involved in several lineage decisions where they induce quite different cell fates depending on the presence of other transcription factors co-expressed in the cell or on the level of protein expression. Therefore, the hematopoietic differentiation process clearly demonstrates the importance of regulatory networks that integrate several intrinsic and extrinsic signals for the outcome of differentiation and cell fate decisions.

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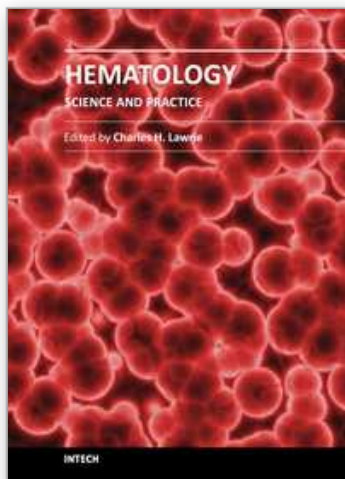
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## **Hematology - Science and Practice**

Edited by Dr. Charles Lawrie

ISBN 978-953-51-0174-1

Hard cover, 596 pages

**Publisher** InTech

**Published online** 02, March, 2012

**Published in print edition** March, 2012

Hematology encompasses the physiology and pathology of blood and of the blood-forming organs. In common with other areas of medicine, the pace of change in hematology has been breathtaking over recent years. There are now many treatment options available to the modern hematologist and, happily, a greatly improved outlook for the vast majority of patients with blood disorders and malignancies. Improvements in the clinic reflect, and in many respects are driven by, advances in our scientific understanding of hematological processes under both normal and disease conditions. Hematology - Science and Practice consists of a selection of essays which aim to inform both specialist and non-specialist readers about some of the latest advances in hematology, in both laboratory and clinic.

### **How to reference**

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Katja Fiedler and Cornelia Brunner (2012). Mechanisms Controlling Hematopoiesis, Hematology - Science and Practice, Dr. Charles Lawrie (Ed.), ISBN: 978-953-51-0174-1, InTech, Available from:

<http://www.intechopen.com/books/hematology-science-and-practice/mechanisms-controlling-hematopoiesis>

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