Networks Establishing Hematopoietic Stem Cell Multipotency and Self-Renewal

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1. Introduction

Hematopoiesis is a tightly regulated process maintained by a small pool of hematopoietic stem cells (HSC) capable of undergoing self-renewal and generating mature progeny of all of the hematopoietic cell lineages. To sustain the proper levels of blood cells, HSCs must continuously monitor and regulate the balance between self-renewal and lineage differentiation. To produce all hematopoietic cells, hematopoiesis proceeds in a step-wise manner from the primordial long-term (LT)-HSCs. LT-HSCs possess the ability to self-renew and the capacity for long-term reconstitution of lethally irradiated hosts. After a first step of differentiation, LT-HSCs lose their capacity for self-renewal and give rise to a population of short-term (ST)-HSCs. The ST-HSCs has a limited ability to self-renew and reconstitute lethally irradiated hosts, but differentiate into a multipotent progenitor (MPP) population. The MPPs lack the capacity to undergo self-renewal, but retain multipotency. From these multipotent progenitors develops a series of intermediate progenitors that give rise to the assorted hematopoietic lineages. In the classical pathway of hematopoiesis, these intermediates include the common lymphoid progenitors (CLPs) that differentiate into lymphoid, but not myeloid progeny, and the common myeloid progenitors (CMPs), which retain full erythromyeloid potential. The CMPs further differentiate to form the granulocyte/macrophage progenitors (GMPs) that differentiate to the myelomonocytic lineage and the megakaryocytic/erythrocyte progenitors (MEPs) that eventually differentiate to form red blood cells and platelets. All these blood cells produced daily in high numbers ($1 \times 10^{12}$ cells/day) are derived from a relatively small but rare fraction of multipotent cells, the HSCs (Weissman, 2000).

Transcriptional regulation is a key mechanism controlling HSC homeostasis, development, and lineage commitment.

2. Transcription factors in hematopoietic development

Hematopoiesis is regulated at the level of pluripotent HSCs and committed progenitors through growth and/or differentiation inducing factors (like EPO, G-CSF, GM-CSF, IL-1, IL-3) that interact with receptors and initiate signal transduction processes that culminate in the activation of new genetic programs. These external stimuli trigger intrinsic determinants of cell fate, the transcription factors which contribute to the reprogramming of HSCs into cell-
lineage restricted pathways of maturation (Zon, 2008). Although the transcription factors involved in hematopoietic development belong to all classes of DNA-binding proteins some of them are involved in the regulation of self-renewal function primarily on HSCs while the others act on MPPs and/or early committed progenitors entering the cell-lineage restricted pathways of differentiation. While transcription factors as MLL, RUNX1, TEL/ETV6, SCL/TAL1 and LMO2 are required for HSC formation and function, others are necessary as key lineage restricted factors acting at the level of early pre-committed progenitors, using key partners that act synergistically or competing to restrict cell-lineage hematopoietic differentiation. GATA-1 and PU-1, for example, physically interact and antagonize with each other to promote either myeloid or erythroid maturation (Rekhtman et al., 1999; Zhang et al., 1999), which means that suppression of GATA-1 expression favors myeloid differentiation while inhibition of PU-1 promotes erythroid maturation. Additional, antagonistic interactions with other transcription factors have also been reported as C/EBPα that antagonizes FOG-1 in eosinophilic differentiation, EKLF antagonizes Fli-1 for erythroid versus megakaryocytic differentiation. Finally, repression of the Pax-5 gene prevented Pro-B cell maturation to B cells, while promoting multi-potentiality into macrophage, T-NKs and dendritic cells (Huntly & Gilliland, 2005).

Transcription factors also interact with other proteins associated with chromatin modification and form active or repressive transcriptional complexes. Knockout of Scl/Tal1 or Lmo2 abrogates hematopoietic development. The precise mechanism through which such transcription activator or repressor complexes regulate the expression of several genes is critical, since the gene expression pattern regulates cell fate decision via cell-lineage restricted maturation. A critical point, however, for all these transcriptional complexes is the concentration of the transcription factor itself and its affinity to other interactive proteins.

Under normal hematopoiesis, several groups of hematopoietic and mature blood cells are generated. Hematopoiesis occurs unidirectionally and commitment from one step to the next occurs irreversibly, suggesting that transcription factors regulate cell fate along the specific cell-lineage pathways irreversibly. This occurs in such a way because intrinsic transcription factor network is coordinated with inputs resulting from external stimuli initiated within the hematopoietic cell niche. The question, however, whether one cell type of progenitors can be reprogrammed into another phenotype at the level of manipulation of transcription factor activation, is a potentially interesting one. Indeed, evidence now indicates that transfection of Gata-1 into CMPs and/or CLPs redirects their commitment to another cell-lineage restricted pathway as megakaryocytic/erythroid. Similarly, pre-T cells can be reprogrammed to myeloid dendritic cells upon PU-1 overexpression (Laiosa et al., 2006; Orkin & Zon, 2008).

3. Ontogeny of HSCs

In vertebrates, the production of blood stem cells is accomplished by the allocation and specification of distinct embryonic cells in a variety of sites that change during development. In mammals, the sequential sites of hematopoiesis include the yolk sac; an area surrounding the dorsal aorta termed the aorta-gonad mesonephros (AGM) region, the fetal liver, and finally the bone marrow. Recently, the placenta has been recognized as an additional site that participates during the AGM to fetal liver period. The properties of HSCs in each site
differ, presumably reflecting diverse niches that support HSC expansion and/or differentiation and intrinsic characteristics of HSCs at each stage. For instance, HSCs present in the fetal liver are in cycle, whereas adult bone marrow HSCs are largely quiescent.

The initial wave of blood production in the mammalian yolk sac is termed “primitive.” The primary function for primitive hematopoiesis is the production of red blood cells that facilitate tissue oxygenation as the embryo undergoes rapid growth. The hallmark of primitive erythroid cells is expression of embryonic globin proteins. The primitive hematopoietic system is transient and rapidly replaced by adult-type hematopoiesis that is termed “definitive”. In mammals, the next site of hematopoietic potential is the AGM region. Hematopoietic cells were first detected in the aorta of the developing pig more than 80 years ago. Morphological examination revealed that a sheet of lateral mesoderm migrates medially, touches endoderm, and then forms a single aorta tube. Clusters of hematopoietic cells subsequently appear in the ventral wall. Similarly, an intraembryonic source of adult HSCs in mice capable of long-term reconstitution of irradiated hosts resides in the AGM region (Muller et al., 1994). At embryonic day 10.5, little HSC activity is detectable, whereas by day 11 engrafting activity is present. Additional hematopoietic activity in the mouse embryo was detected subsequently in other sites, including the umbilical arteries and the allantois in which hematopoietic and endothelial cells are co-localized (Inman & Downs, 2007). Umbilical veins lack hematopoietic potential, suggesting that a hierarchy exists during definitive hematopoiesis in which HSCs arise predominantly during artery specification. In addition, significant numbers of HSCs are found in the mouse placenta (Gekas et al., 2005; Ottersbach & Dzierzak, 2005), nearly coincident with the appearance of HSCs in the AGM region and for several days thereafter. Placental HSCs could arise through de novo generation or colonization upon circulation, or both. The relative contribution of each of the above sites to the final pool of adult HSCs remains largely unknown.

Subsequent definitive hematopoiesis involves the colonization of the fetal liver, thymus, spleen, and ultimately the bone marrow. It is believed that none of these sites is accompanied by de novo HSC generation. Rather, their niches support expansion of populations of HSCs that migrate to these new sites. However, until very recently, there has been no evidence by fate mapping or direct visualization that HSCs from one site colonize subsequent sites.

4. Pathways involved in the emergence of HSCs

The AGM has been characterized largely by morphology and functional assays, but the pathways involved in HSC generation remain incompletely defined. Studies of chick embryos demonstrate that endoderm has a prominent role and secretes inducing factors. Somitic mesoderm also contributes to the dorsal aspect of the aorta, and the addition of factors such as VEGF, TGF-β, and FGF to the somitic mesoderm leads to induction of hematopoietic tissue. In contrast, TGF-α and EGF suppressed formation of hematopoietic cells (Pardanaud & Dieterlen-Lievre, 1999).

Signaling pathways that regulate the induction of the AGM have been uncovered in mouse and zebrafish, Notch 1 is required for artery identity and aortic HSC production (Kumano et al., 2003). The fate decisions imposed on mesodermal progenitors within the AGM are
clearly influenced by the Notch pathway (Burns et al., 2005). For instance, mice deficient in RBPj (a downstream component of the Notch pathway) show expanded VE-Cadherin and CD31/PECAM endothelial cell expression with concomitant loss of definitive HSCs (Robert-Moreno et al., 2005). Ablation of the COUP-TFII transcription factor in endothelial cells enabled veins to acquire arterial characteristics, including the expression of Notch1 and the formation of ectopic HSCs (You et al., 2005). This result would favor Notch acting to induce HSCs from a hemogenic endothelial cell. The model in which the Notch pathway regulates arterial and HSC fate choice either from distinct mesodermal populations or over different developmental windows since each decision can be uncoupled in vivo is very attractive. The finding that both aorta and vein express HSC markers in the Notch-activated state with minimal change in ephrinB2a expression indicates that Notch independently regulates mesoderm–HSC and artery–vein cell fate decisions. Lateral inhibition has been proposed in the central nervous system whereby Notch signaling promotes non-neural fates while inhibiting neural development (Lewis, 1998). HSC fate may be established by a similar mechanism whereby Notch activation in an endothelial or mesenchymal cell causes down-regulation of ligand production. Consequently, a cell that produces more ligand will force its neighbor to produce less, thus generating a salt-and-pepper pattern of cells containing elevated Notch activity. In this model, cells containing high levels of Notch Intra Cytoplasmatic Domain (NICD) would become HSCs, while those with low NICD activity would remain endothelial or mesenchymal.

5. Hematopoietic niches

Stem cells depend on their microenvironment, the niche, for regulation of self-renewal and differentiation. As the site of hematopoiesis changes during vertebrate development, the nature of the stem cell niche must also change. Mutant mice in which the BMP pathway is disrupted have increased numbers of osteoblasts and HSCs (Calvi et al., 2003; Zhang et al., 2003). These findings suggest that osteoblasts may represent a critical component of the bone marrow niche for HSCs. Microscopical examination revealed that HSCs appear to reside in the periosteal area of calvarium marrow, where osteoblasts represent an essential component of the bone marrow niche (Papadimitriou et al., 1994). Most recent live animal tracking experiments by using real-time imaging of individual HSCs have indicated that endosteum forms a special zone where HSCs reside (Lo Celso et al., 2009; Xie et al., 2009). The bone marrow HSC niche is constituted of mesenchymal cells type osteoblasts, extracellular matrix components and minerals (high density calcium salts), all of which contribute to the unique micro-environment (niche) (Moore & Lemischka, 2006; Wilson & Trumpp, 2006). At least two distinct hematopoietic progenitor cell supportive niches in bone marrow have been identified thus far: the osteoblastic, which is regulated by BMP, osteopontin, angiopoietin-1, notch and maybe others (Adams & Scadden, 2006; Wilson & Trumpp, 2006) and the other one, the vascular niche. The vascular niche is thought to be the site where actively dividing stem or progenitor cells is located, and osteoblastic niche is an environment promoting maintenance of quiescent HSCs (Calvi et al., 2003). Currently, how these two different niches communicate with each other is largely unknown.

The number of HSCs in the bone marrow niche is highly controlled through physical interactions among different cell types, in a way that maintains stem cell state. HSCs remain in a quiescent state through close interaction with osteoblasts where this interaction is not
only crucial to attach HSCs to niche osteoblasts, but is also essential to maintain HSC dormancy and function. Many factors, including ligands for Notch receptors and N-cadherin, are liberated by osteoblasts, although the contribution of these to adult hematopoiesis remains to be established. The role of N-cadherin as a mediator of interactions with osteoblasts (Zhang et al., 2003), as well as the prominence of osteoblasts for HSC adherence, has been challenged (Kiel et al., 2007). Recent findings suggest that HSCs are maintained in a quiescent state through interaction with thrombopoietin-producing osteoblasts (Yoshihara et al., 2007). Thrombopoietin (TPO) is the primary cytokine that regulates megakaryocyte and platelet development. Thrombopoietin and its receptor Mpl also exert profound effects on primitive hematopoietic cells. All HSCs express Mpl; TPO−/− or Mpl−/− mice have a decreased number of repopulating HSCs (Solar et al., 1998). In vitro culture studies (Matsunaga et al., 1998) also indicate a role of TPO in promoting the survival of repopulating HSCs. Through study of AGM and fetal liver Mpl−/− HSCs, Petit-Cocault et al. (2007) showed that TPO contributes to both generation and expansion of HSCs during definitive hematopoiesis. An intracellular adaptor, Lnk, induces a negative signaling pathway downstream of TPO in HSCs (Buza-Vidas et al., 2006; Seita et al., 2007). Another study (Tong et al., 2007) on mice that express Mpl lacking the C-terminal 60 amino acids revealed a pivotal role of an unknown signal emanating from the membrane proximal region of the Mpl receptor or from JAK2 that is critical for maintenance of HSC activity. The association of HSCs with osteoblasts is countered by other studies that place HSCs adjacent to vascular cells. The chemokine CXCL12 regulates the migration of HSCs to the vascular cells (Kiel & Morrison, 2006). Taken together, these findings suggest that HSCs reside in various sites within the marrow and that their function might depend on their precise localization. Much of the existing debate may be semantic, however, if the osteoblastic and vascular niches are intertwined and not physically separate. Alternatively, HSCs may truly reside in distinct sub-regions, which may endow them with different activities. Cellular dynamics within the niche are relevant to clinical marrow transplantation. For example, recent findings suggest that antibody-mediated clearance of host HSCs facilitates occupancy of the niche and transplantation by exogenous HSCs (Czechowicz et al., 2007).

The physical interactions between individual HSCs and osteoblasts may be effective in determining the stem cell number by facilitating asymmetric or symmetric divisions, which in turn enable HSCs to either self-renew themselves or give birth to early progenitors for blood cells production (Moore & Lemischka, 2006; Wilson and Trumpp, 2006). HSCs are not of static nature, but exist in a dynamic state, since they migrate from the bone marrow into the peripheral blood (frequent trafficking). Whether, or not, HSCs contribute into the repair of the vascular system, is still not known (Janzen & Scadden, 2006).

5.1 Signaling in the niche

Many cell culture experiments have shown that HSCs respond to multiple cytokines and that the fate of a HSC self renewal, apoptosis, mobilization from the niche, formation of differentiated progeny cells depends on multiple cytokines, adhesion proteins, and other signals produced by stromal cells and likely other cells in the body. Since osteoblast (a cell derived from mesenchymal stem cells) is a key component in the HSC niche for the
regulation of HSC number via self-renewal (Adams & Scadden, 2006; Huang et al., 2007), modifications of osteoblast functions in co-orchestration with other niche components, would be pivotal for HSCs survival, self-renewal, differentiation and apoptosis under certain circumstances.

HSCs fate decisions is activated by external environmental stimuli and coordinated by intrinsic factors. External stimuli include hematopoietic growth factors such as SCF, BMP/TGF-β, FGF, TPO, WNT proteins (WNT3A), Angiopoietin-1, IL-3, IL-6, Flt3-ligand, as well as Ca2+, hypoxia, PGE2 and retinoic acid (Wilson & Trumpp, 2006) while intrinsic factors are essentially genes controlling cell cycle, apoptosis and chromatin remodeling.

5.2 How some extrinsic factors act in the niche

Stem cell factor receptor, also known as c-kit and its ligand SCF play a central role in hematopoiesis, melanogenesis and gametogenesis (Edling & Hallberg, 2007; Kent et al., 2008). C-kit is a member of the type-III subfamily of receptor tyrosine kinases that also includes the receptor for M-CSF, Flt-3 and PDGF. It is expressed in HSCs (LT-HSCs, ST-HSCs and MPPs) (Zayas et al., 2008), normal B- and T-cell progenitors, mast cells, germ cells, melanocytes, neurons, glial cells, placenta, kidney, lung and gut cells. Deficiency and/or deregulation in SCF or c-kit produce defects in hematopoiesis leading to Acute Myeloid Leukemia (AML) (Scholl et al., 2008). Sporadic mutations of c-kit and autocrine/paracrine activation pathways of the SCF/c-kit pathway have been implicated in a variety of malignancies. Gain of function mutations of c-kit are associated with malignancies such AML, gastrointestinal tumors and mastocytomas. Moreover, expression of a defective c-kit leads to a decrease in repopulating HSCs (Ikuta & Weissman, 1992).

Binding of SCF to c-kit promotes dimerization and activation of protein kinase that auto-phosphorylates the receptor. Although SCF may not be essential for the generation of HSCs, numerous studies have shown that it prevents HSC apoptosis. Almost all cytokine combinations used to date for culturing HSCs include SCF. SCF potentiates the greater ability of fetal liver HSCs than adult HSCs to undergo symmetric self-renewal in culture this activity likely needs the cooperation of other factors. The membrane-bound form of SCF is also an adhesive molecule for HSCs to the bone marrow environment (Heissig et al., 2002) as interruption of the interaction between the membrane-bound stem cell factor on osteoblasts with the c-kit receptor on HSCs by blocking antibodies has demonstrated that c-kit signaling is essential to maintain HSC dormancy and function (Suzuki et al., 2006), and an increased number of osteoclasts was associated with HSC mobilization. Receptor activator of nuclear factor (NF)-κB (RANK) ligand and cathepsin K mediate the cleavage of membrane-bound SCF; this decreases the abundance of SCF and, therefore, increases HSC mobilization (Kollet et al., 2006). The involvement of SCF in survival, mobility and possibly self-renewal of HSCs in culture and in the HSC niche likely reflects the complex relationship of different cell fates of HSCs.

Transforming growth factor (TGF)-β potently inhibits HSC activity in vitro (Blank et al., 2008). However, a TGF-β signaling deficiency in vivo does not affect proliferation of HSCs. TGF-β and BMP are secreted ligands that are recognized by different receptors that dimerizes and activates downstream cytosolic targets, culminating with the translocation of these activated transcription factors to the nucleus. BMPs, members of the TGF-β
superfamily, play important roles in HSC specification during development. A negative role of BMP signaling in maintenance of mouse HSCs was shown by its control of the size of the HSC endosteal niche (Ross & Li, 2006). BMP4 supports HSC expansion in culture and partially mediates the effects of Sonic hedgehog on cultured human HSCs (Bhardwaj et al., 2001). Recently, expression characterization of TGF-β superfamily ligands, receptors, and Smads in mouse HSCs was published; primary HSCs and the Lhx2-HPC cell line express most of the proteins required to transmit signals from several TGF-β family ligands (Utsugisawa et al., 2006). In addition, Pimanda et al. (2007) demonstrated the integration of BMP4/Smad pathway and Scl and Runx1 activity in HSC development.

All long-term repopulating bone marrow HSCs express a fibroblast growth factor (FGF) receptor (Yeoh et al., 2006); both FGF-1 and FGF-2 support HSC expansion when unfractionated mouse bone marrow cells are cultured in serum-free medium. Careva et al. (2005) confirmed that FGF-1 stimulates ex vivo expansion of HSCs and showed that the expanded cells were efficiently transduced by retrovirus vectors. Conditional derivatives of FGF receptor-1 have also been used to support short-term HSC expansion and long-term HSC survival in culture (Schiedlmeier et al, 2007). However, the role of the FGF pathway in regulating adult HSCs or embryonic hematopoietic development is controversial as the same authors showed that the treatment of purified mouse HSCs that ectopically express HoxB4 with the fibroblast growth factor receptor (FGFR) inhibitor SU5402 enhanced HSC repopulating activity. Similar results were obtained using primitive hematopoietic colonies derived from embryonic stem cells. These inconsistent results were obtained from different starting cell populations and under different culture conditions, suggesting that the crosstalk of FGF signaling with other pathways is complex.

The WNT protein binds to a receptor complex consisting of a member of the Frizzled family of seven transmembrane proteins and the LDL receptor-related proteins LRP5 or LRP6 (Clevers, 2006). In the canonical Wnt pathway, receptor activation leads to stabilization of β-catenin, which accumulates and translocates to the nucleus where it activates target gene expression in concert with transcription factors such as TCF and LEF. Fleming et al. (2008) analyzed the role of Wnt signaling on HSC activity, including its effects on cell-cycle quiescence and the capacity of HSCs to reconstitute the hematopoietic system of recipient mice (whose bone marrow has been ablated by radiation). In contrast to previous studies that genetically manipulated the HSCs themselves, they analyzed the effects of blocking Wnt signaling in the mouse bone marrow microenvironment by overexpression of dickkopf1 (Dkk1), an antagonist of Wnt/β-catenin signaling. Dkk1 is a soluble secreted protein that interacts with the Wnt co-receptors LRP5 and LRP6 (Kawano & Kypta, 2003). It is known that the number of osteoblasts directly affects the number of long-term repopulating HSCs (Calvi et al., 2003; Zhang et al., 2003). The overexpression of Dkk1 in the osteoblastic lineage under the control of the 2.3 kb fragment of the collagen 1α promoter reduced activation of the Tcf/lef transcription factors in HSCs in a non-cell-autonomous manner.

The transgenic mice showed no significant alteration in the proportion of HSCs and common lymphoid progenitor cells under steady-state conditions. Although HSCs from the Dkk1 transgenic mice could reconstitute the hematopoietic system of irradiated recipient mice, they lost their reconstituting capacity after repeated bone marrow transplantation, indicating that the inhibition of Wnt signaling in the niche results in the premature loss of
self-renewal activity. These findings show that Wnt/β-catenin activity is crucial for the maintenance of HSC quiescence in the bone marrow niche.

The angiopoietin (Ang) family of growth factors is composed of four members that bind to the Tie-2 tyrosine kinase receptor; Ang growth factors are important modulators of angiogenesis. Members of the angiopoietin family of proteins contain an N-terminal coiled-coil domain that mediates homo-oligomerization and a C-terminal fibrinogen-like domain that binds Tie-2. To identify the HSCs in situ, Arai et al. (2004) analyzed the receptor tyrosine kinase Tie-2 expression in bone marrow and found that 5-FU-resistant Tie-2 expressing HSCs adhere to osteoblasts at the endosteal surface, in agreement with previous findings of Calvi et al. (2003) and Zhang et al. (2003). They also demonstrated that angiopoietin-1 (Ang-1), a Tie-2 receptor ligand, is produced primarily by osteoblasts, indicating that Tie-2 and Ang-1 are expressed complementarily in the niche. Tie-2 together with Tie-1 was also found required for homing of HSCs to bone marrow. Taken together, Tie receptors seem one group of the likely candidates for localizing stem cells to the stem cell niche.

Mineral content of bone contributes to compose a unique extracellular matrix in bone marrow and distinguishes it from other mesenchymal tissues. The extracellular calcium concentrations are recognized by the seven-transmembrane calcium-sensing receptors and therefore can initiate an intracellular G protein-coupled response. Those receptors are found on hematopoietic cells and have also been identified on the surface of HSCs (Adams et al., 2006). Local calcium gradient is involved in retaining HSCs in close physical proximity to the endosteal surface of bone. Extracellular calcium ion concentrations in the endosteum are likely higher than in the central marrow region (Silver et al., 1988). In receptor deficient mice models, HSCs were found not to engraft in the bone marrow (Adams et al., 2006) suggesting that the ability of stem cells to sense and respond to the increased calcium concentrations at the endosteal surface participates in creating the unique stem cell-niche interaction that enables bone marrow hematopoiesis.

Most slow-cycling hematopoietic cells are found in the hypoxic zones close to bone surface and distant from capillaries (Kubota et al., 2008), raising the possibility that these hypoxic niches are important for diminished HSC proliferation. Evidence for quiescent HSCs situated in a hypoxic environment has lately been confirmed by analyzing bone marrow cells from mice injected with a Hoechst dye. Transplantation results showed that the bone marrow fraction with the lowest Hoechst-dye uptake, inferred to be hypoxic, had the highest amount of long-term repopulating cells (Parmar et al., 2007). Consistently, HSCs were found to be the most positive for binding of the hypoxic probe pimonidazole. The molecular mechanisms involve the hypoxia-inducible factor-1α regulated gene expressions in stromal cells, such as c-Kit, stromal cell derived factor-1, and others (Ceradini et al., 2004).

Other molecules were recently identified to have role in signaling pathways inside the niche. DNA array experiments showed that, among other proteins, IGF-2 is specifically expressed in cells that do support HSC expansion in culture. Moreover, it was showed that all fetal liver and bone marrow HSCs express receptors for IGF-2. The inclusion of IGF-2 with SCF, TPO, and FGF-1 supports an eight-fold increase of highly enriched HSCs in culture (Zhang & Lodish, 2004). Whether IGF-2 acts on self-renewal, apoptosis,
differentiation, or homing of HSCs is unclear. Interestingly, IGF-2 was found to bind and stimulate self-renewal of human embryonic stem cells (Bendall et al., 2007). Angiopoietin-like proteins (Angptls) were also implicated in HSC expansion. Angptls are a family of seven secreted glycoproteins that share sequence homology with the angiopoietins (Morisada et al., 2006). Similar to the angiopoietins, each Angptl contains an N-terminal coiledcoil domain and a C-terminal fibrinogen-like domain. However, unlike angiopoietins, Angptls do not bind to Tie-2 or Tie-1 and their receptors are unknown. This suggests that Angptls may have different functions from the angiopoietins. Angptl7 was suggested to be a target of the Wnt/β-catenin signaling pathway. However, most of the physiological activities of the Angptls remain unknown. Recently Angptl2 and Angptl3 were identified as growth factors that stimulate \textit{ex vivo} expansion of bone marrow HSCs. Other analogues, including Angptl5, Angptl7, and Mfap4, also support \textit{ex vivo} expansion of HSCs.

5.3 Signaling through cell adhesion molecules

In addition to signaling pathways as described above, extracellular matrix components of the niche have also been shown to play role in regulating the HSC dynamics. A matrix glycoprotein, osteopontin (OPN), as a constraining factor on HSCs within the bone marrow microenvironment is produced by osteoblasts in response to stimulation (Stier et al., 2005). Using studies that combine OPN-deficient mice and exogenous OPN, Stier et al. (2005) demonstrated that OPN modifies primitive hematopoietic cell number and function in a stem cell non-autonomous manner. The OPN-null microenvironment is sufficient to increase the number of stem cells associated with increased stromal \textit{Jagged-1} and \textit{Ang-1} expression and reduced primitive hematopoietic cell apoptosis. The activation of the stem cell microenvironment with PTH was shown to induce a super-physiologic increase in stem cells in the absence of OPN. Therefore, OPN seems to be a negative regulatory element of the stem cell niche that limits the size of the stem cell pool and may provide a mechanism for restricting excess stem cell expansion under conditions of niche stimulation.

The production of OPN by osteoblasts is likely to be an essential requirement as shown by Karahuseyinoglu et al. (2007). Osteogenically induced umbilical cord stromal cells express OPN during the first week of induction followed by a third week expression of another matricellular protein, bone sialoprotein-2 (BSP-2). In the following weeks, in conditioned media differentiating osteoblasts express osteonectin and osteocalcin that led us to suggest that all those proteins have roles in autocrine regulation of osteoblast maturation and thus might serve to determine the conditional status of the partner cell(s) in hematopoietic niche microenvironment.

Previous studies showed that cell adhesion molecules, such as cadherins and integrins, are crucial for the interactions between HSCs and the osteoblastic niche. N-cadherin-mediated adhesion mediates slowing cell cycling of HSCs and may keep HSCs quiescent. Some studies showed that specialized spindle-shaped N-cadherin* osteoblasts are a key component of the bone marrow stem cell niche. HSCs are thought to be anchored to spindle-shaped N-cadherin* osteoblast cells via a homotypic N-cadherin interaction. Also, N-cadherin and β1-integrin are identified as the downstream targets in Tie-2/Ang-1 signaling and TPO/MPL signaling (Yoshihara et al., 2007) in HSCs, respectively, suggesting a link between adhesion molecules and cell-cycle regulators in modulating the HSC–niche interaction. These data suggest cell-adhesion molecules not only contribute to the anchoring
of HSCs to the niche, but also regulate cell-cycle quiescence of HSCs in the niche. However, the studies by conditional deletion of N-cadherin fail to support the effects of N-cadherin on hematopoiesis (Kiel et al., 2007).

The members of the Notch family are developmental morphogens shown to be expressed in self-renewing tissues, enhance the self-renewal capacity of HSCs and promote T-cell differentiation. Notch signaling is initiated by the involvement of the extracellular portion of Notch with its ligands Jagged/Delta. Activation of the Notch signaling pathway has been shown to potentiate self-renewal of HSCs. It is initiated by the binding of Jagged ligand to Notch protein followed by metalloproteinase (γ-secretase) cleavage in the extracellular receptor portion leading to the intracellular release of Notch (NICD). Then Notch translocate into the nucleus, where it forms a multimeric transcriptional complex with other transcription factors (Huntly & Gilliland, 2005). Inhibitors of γ-secretase abrogate the Notch signaling activation (Rizzo et al., 2008; Shih & Wang, 2007).

Calvi et al. (2003) and Duncan et al. (2005) demonstrated that the Notch signaling pathway plays a role in the osteoblast bone marrow HSCs niche. Notch ligands have positive effects on ex vivo expansion of HSCs: activated Notch is able to immortalize primitive mouse hematopoietic progenitors and Notch ligands support HSC expansion in culture (Chiba, 2006). Recently, by culturing human cord blood cells in serum-free medium supplemented with SCF, TPO, Flt3L, IL-3, IL-6/sIL-6R, and Delta 1, Suzuki et al. (Suzuki et al., 2006) reported an approximate six-fold increase in SCID-repopulating cell (SRC) number. It is noteworthy that there exists a dose effect for Notch ligands in HSC culture. Whereas a low amount of Delta 1 supports human cord blood SRC expansion, high amounts of the cytokine induce apoptosis (Chiba, 2006).

This emphasizes the complicated relationship among the different fates of HSCs. As conditional knockouts of Notch1 and Jagged1 have normal in vivo HSC activities (Mancini et al., 2005), there likely is functional redundancy of different Notch isoforms and their ligands.

Endothelial cells in the vascular niche environment contacting HSCs also provide maintenance signals on the HSC behaviour (Coultas et al., 2005; Li & Li, 2006). The main components of vascular niche - hematopoietic cells and endothelial cells - are closely related during development since they are both derived from haemangioblasts (Kopp et al., 2005). Previous studies have suggested that the vascular niche is the place for HSC differentiation and mobilization (Avencilla et al., 2004). Endothelial cells expressing vascular cell-adhesion molecule-1 (VCAM-1) associate closely with megakaryocytes and their progenitors through VLA-4 in response to chemotactic factors, stromal cell-derived factor-1 (SDF1) and fibroblast growth factor-4 (FGF4), and thus provide a niche for megakaryocyte maturation and platelet production. The immediate juxtaposition of HSCs to endothelial cells also facilitates their rapid mobilization and entry into circulation in response to stress and, in the case of megakaryocytes, release of platelets directly into the blood. Endothelial cells promote survival of HSCs in culture, but this seems to be limited to certain populations of endothelial cells (Li et al., 2004). Fractions of HSCs in both adult bone marrow and spleen were found in close association with endothelial sinusoids (Kiel et al., 2005), suggesting that endothelial cells provide support to HSCs in vivo. Depending on these data, it is now plausible to note that while the osteoblastic niche provides a quiescent environment for HSC maintenance, the vascular
niche offers an alternative niche for mobilized stem cells and promotes proliferation and further differentiation or maturation into the circulatory system. It would be interesting to further define the respective contributions of endothelial and endosteal niches to HSC behaviour.

5.4 Cell intrinsic responses

Recent studies have shown that Polycomb group (PcG) proteins and their interaction are important in the regulation of HSC self-renewal and lineage restriction. In particular, members of the PRC1 (Polycomb repression complex 1), such as Bmi1, Mel18 and Rae28, have been implicated. Bmi1 plays an important role in regulating the proliferative activity of stem and progenitor cells. It is required for the self-renewal of both adult HSCs and neural stem cells (Molofsky et al., 2005; Park et al, 2003). Bmi1 enhances symmetrical expansion of the stem cell pool through self-renewal, induces a marked ex vivo expansion of multipotent progenitors, and increases the ability of HSCs to repopulate bone marrow in vivo (Iwama et al., 2004). Leukemic cells lacking Bmi1 undergo proliferation arrest, differentiation and apoptosis, leading to failure of leukemia in a mouse transplant model (Lessard & Sauvageau, 2003). In Bmi1-deficient bone marrow there is an up-regulation of cell cycle inhibitors p16 and p19, and the p53-induced gene Wig1, and a down-regulation of the apoptosis inhibitor AI-6. This suggests that a mechanism exists whereby Bmi1 functions by modulating proliferation and preventing apoptosis (Park et al., 2004). Bmi1 has also been shown to regulate the expression of Hox genes that are required for differentiation during hematopoiesis (van der Lugt et al., 1996). Loss or knockdown of another Polycomb gene, Mel18, leads to increased expression of Hoxb4 (Kajiume et al., 2004), and transplanted Mel18-deficient bone marrow showed an increase in overall HSC numbers but a decrease in their activity owing to arrest in G0 phase of the cell cycle. Rae28-deficient HSCs were defective in their long-term repopulating ability in serial transplantation experiments (Kim et al., 2004; Ohta et al., 2002). Taken together, these studies show the importance of the Polycomb proteins in HSC self-renewal and maintenance of the blood system.

Transcriptional repression by PcG proteins is essential for maintenance of HSC identity. Part of the mechanism by which it functions is by repression of genes that promote lineage specification, cell death and cell cycle arrest. More recently, PcG complexes have been shown to be essential for maintenance of the undifferentiated state in murine embryonic stem (ES) cells and human ES cells by directly repressing a large number of developmental regulators (Boyer et al., 2006; Lee et al., 2006). PcG complexes bind to and presumably repress the expression of a subset of these genes linked to differentiation. This represents a dynamic repression of genes required for differentiation, and a scenario in which PcG proteins act as transcription repressors by cooperating with a specific set of transcription factors in stem cells. Some target genes include Hox family members important for induction of differentiation. Expression of Hox genes that are involved in differentiation is repressed in the ES cells by PcG proteins. Thus, PcG complex repression is also necessary for ES cell identity. Taken together, these studies suggest that differentiation is the default state during stem cell replication, and self-renewal requires active repression of transcription factors that prevent self-renewal.
The transcription factor Tel (Translocation Ets leukemia; also known as Etv6 [Ets variant gene 6]), an Ets (E-26 transforming-specific)-related transcriptional repressor, is also required for HSC maintenance. Conditional inactivation of Tel/Etv6 in HSCs rapidly leads to the depletion of Tel/Etv6-deficient bone marrow. However, Tel/Etv6 is not required for the maintenance of committed precursors. When it is conditionally inactivated in most hematopoietic lineages, it does not affect their differentiation or survival (Hock et al., 2004). At the moment, the mechanism by which Tel/Etv6 modulates adult HSCs renewal is not known. Study of the downstream targets it represses should shed light on other players essential for HSC maintenance.

Pbx1 (pre-B-cell acute lymphoblastic leukemia) is a TALE class homeodomain transcription factor that critically regulates numerous embryonic processes, including hematopoiesis. Pbx1 is preferentially expressed in LT-HSCs compared to more mature short-term HSCs and multipotent progenitor cells (Ficara et al., 2008). By using Pbx1-conditional knockout mice, it was revealed that Pbx1 positively regulates HSC quiescence. Transcriptional profiling showed that a significant proportion of Pbx1-dependent genes are associated with the TGF-β pathway.

The homeobox (Hox) genes encode transcription factors that regulate embryonic body patterning and organogenesis. They play a role in the regulation of hematopoiesis. Overexpression of HoxB4 in bone marrow leads to expansion of HSCs in vivo and in vitro, therefore appearing to be a positive regulator of HSC self-renewal (Antonchuck et al., 2002; Krosl et al., 2003; Miyake et al., 2006; Sauvageau et al., 1995). It therefore came as a surprise when HoxB4-deficient mice had normal hematopoietic development but exhibited only mild proliferative HSC defects (Brun et al., 2004). In an attempt to determine if this was due to compensatory mechanisms, the entire HoxB cluster was deleted. However, this did not lead to major defects in hematopoiesis (Bijl et al., 2006), possibly owing to compensation by HoxA4 and/or HoxC4.

Gfi1 (Growth factor independence 1), a zinc-finger repressor, has been recently implicated as a regulator of HSC self-renewal. Two groups working independently determined that Gfi1 controls self-renewal of HSCs by restraining their proliferative potential (Hock et al 2004; Zeng et al., 2004). They showed that Gfi1-deficient HSCs display increased proliferation rates and are also functionally compromised in competitive repopulation and serial transplantation assays. Gfi1 might exert its effects on HSC proliferation by regulating the cell cycle inhibitor p21.

Gfi1 is originally recognized for its role in T-cell differentiation and lymphoma. Gfi1 gene knockout is one of the first targeted mutants to exhibit the combination of an increase in cycling HSCs at the expense of HSC function. Both Gfi1 knockout models displayed an increase in cycling cells within the HSC pool, a large decrease in HSC function in transplantation experiments. Profoundly reduced expression of p21, the cyclin-dependent kinase inhibitor, in Gfi1 null HSCs may account for the mechanism. Thus, under normal homeostasis, Gfi1 is thought to suppress the proliferation of HSCs, thereby keeping HSCs in quiescence.

Numerous studies have identified roles for p53 in the proliferation, differentiation, apoptosis, and aging of hematopoietic cells. LT-HSCs express high levels of p53 transcripts, which is an indication of roles of p53 in HSC physiology (Dumble et al., 2007). Recently, p53
has been identified as a positive regulator of HSC quiescence through analysis of $p53^{-/-}$ mice (Liu et al., 2009). Furthermore, in the same study, it was demonstrated that the increased quiescence of HSCs from MEF null mice, in which both $p53$ and $p21$ are up-regulated, is dependent on $p53$, but not $p21$, further confirming the positive role of $p53$ in maintaining HSC in quiescence. Gfi1 was identified as $p53$ target gene, which is both shown important in regulating HSC quiescence by up-regulation or knockdown experiments.

Stem cell leukemia/T-cell acute lymphoblastic leukemia 1 ($SCL/TAL1$) plays a key role in controlling development of primitive and definitive hematopoiesis during mouse development. In adult HSCs, it is highly expressed in LT-HSCs compared with short-term HSCs and progenitors (Lacombe et al., 2010). SCL impedes $G_0-G_1$ transition in HSCs. The function of HSCs from $Scl^{-/-}$ mice or with decreased dosage of SCL protein by in vitro interference was shown decreased in various transplantation assays. At the molecular level, SCL maintains HSC quiescence by regulating gene expression of $Cdkn1a$ and $Id1$.

Recently, many other transcriptional factors, such as interferon regulatory factor−2, a transcriptional suppressor of interferon signaling (Sato et al., 2009); Nurr1, a nuclear receptor transcription factor (Sirin et al., 2010); and thioredoxin-interacting protein, a transcriptional repressor (Shao et al., 2010), have been identified as positive regulators of HSC quiescence. Loss of HSC quiescence was observed in mice with deletion of each of these factors.

Individual member of Retinoblastoma ($Rb$) tumor suppressor gene family serves critical roles in the control of cellular proliferation and differentiation with functional redundancy for each other. The mice with conditional triple knockout of $Rb$ family genes including $Rb$, $p107$, and $p130$ display a cell-intrinsic myeloproliferation that originates from hyperproliferative early hematopoietic progenitors due to the loss of quiescence, and the mutant HSCs show strong short-term repopulation capacity but impaired long-term repopulation ability on transplantation. Thus, $Rb$ family members collectively maintain HSC quiescence (Viatour et al., 2008).

It has been shown that the conditional inactivation of $c-Myc$ induces excessive expression of integrins and $N-cadherin$ in HSCs, leading to the enhanced HSC interaction with the niche, which subsequently enable Myc-deficient HSCs stay in quiescence. Conversely, enforced $c-Myc$ expression in HSCs downregulates $N-cadherin$ and integrins, leading to a loss of HSC function (Wilson et al., 2004).

$p21$ mRNA expression levels are dramatically lower in the Gfi1-deficient HSCs. $p21$ itself has been implicated in the regulation of HSCs (Cheng et al., 2000). In its absence, HSCs have an impaired serial transplantation capacity. Another cell cycle inhibitor, $p18$, has also been shown to affect HSC self-renewal. The absence of $p18$ leads to increased HSC self-renewal (Yuan et al., 2004; Yu et al., 2006). Therefore, intricate control of the cell cycle and proliferation machinery is required for self-renewal regulation.

In contrast to $p21$, little is known about the role of $p57$ in adult stem cell populations. Using primary human hematopoietic cells and microarray analysis, Scandura et al. (2004) identified $p57$ as the only cyclin-dependent kinase inhibitor induced by TGF-β. Up-regulation of $p57$ is essential for TGF-β–induced cell-cycle arrest in these cells, which may represent the mechanisms by which TGF-β affects cell-cycle arrest and stem cell quiescence.
Bone marrow is a very low oxygen tension environment that would protect cells from exposure to oxidative stress. Various intrinsic factors have also been identified to function in maintaining low oxidant levels in HSCs. ATM, a cell-cycle checkpoint regulator activated after DNA damage, is shown to regulate oxidant levels in HSCs (Ito et al., 2006). ATM deficiency-induced ROS elevation in HSCs specifically activates the p38 mitogen-activated protein kinase (MAPK) pathway, a signaling pathway responding to diverse cellular stresses, leading to a defect in the maintenance of HSC quiescence (Ito et al., 2004). ATM−/− mice over the age of 24 weeks show progressive bone marrow failure due to a defect in HSC function associated with elevated levels of ROS. Treatment with anti-oxidative reagents, N-acetyl cysteine or with a MAPK inhibitor restores reconstitutive capacity and quiescence of ATM−/− HSCs.

Members of the FoxO subfamily of forkhead transcription factors have been shown to protect HSCs from oxidative stress by up-regulating genes involved in their detoxification. Triple knockout mice of FoxO1, FoxO3, and FoxO4 exhibited defective long-term repopulating activity of HSCs, which correlated with increased cycling and apoptosis of HSCs, as well as increased levels of ROS in HSCs (Tothova et al., 2007). Similarly, the HSC compartment in FoxO3a null mice suffers from augmented levels of ROS and subsequent bone marrow failure (Miyamoto et al., 2007). The HSC defect resulting from loss of FoxOs could also be rescued by administration of the antioxidant N-acetyl cysteine.

It is conceivable that both the hypoxic environment in which the HSCs reside and the intrinsic factors in HSCs serve to protect HSCs from oxygen radicals, keeping HSCs’ quiescent status.

The JAK–STAT (Janus family kinase–signal transducer and activator of transcription) pathway is a common downstream pathway of cytokine signaling that promotes hematopoiesis. Constitutive activation of the transcription factors of the Stat family, particularly Stat3 and Stat5, are frequently detected in leukemias, lymphomas and solid tumors. In order to evaluate their role in HSCs, constitutively active Stat mutants were used to activate signaling in HSCs. Activation of Stat5 in HSCs led to the dramatic expansion of multipotent progenitors and promoted HSC self-renewal ex vivo (Kato et al., 2005). Deletion of Stat5 resulted in profound defects in hematopoiesis and markedly reduced ability of the mutant cells to repopulate the bone marrow of lethally irradiated mice (Snow et al., 2002). In a mouse model of myeloproliferative disease (MPD), sustained Stat5 activation in HSCs and not multipotent progenitors induced fatal MPD, suggesting that the capacity of Stat5 to promote self-renewal of hematopoietic stem cells is crucial for MPD development. Another group showed that transduction of adult mouse bone marrow cells with a constitutively activated form of Stat3 increased their regenerative activity in lethally irradiated recipients, whereas the transduction of these cells with a dominant negative form of Stat3 suppressed their regenerative activity (Chung et al., 2006). These studies suggest that Stat proteins play a role in HSC self-renewal and potentially in other tissues; owing to the wide range of solid tissue and blood malignancies that harbor constitutively activated Stats.

Studies using transgenic mice constitutively expressing BCL2 (B-cell lymphoma 2) in all hematopoietic tissues provide evidence directly supporting this theory. The forced
expression of the oncogene Bcl2 resulted in increased numbers of transgenic HSCs in vivo and gave these cells a competitive edge over wild type HSCs in competitive reconstitution experiments (Domen et al., 1998; Domen et al., 2000) suggesting that cell death plays a role in regulating the homeostasis of HSCs. Recently, Mcl1 (Myeloid cell leukemia 1), another anti-apoptotic Bcl2 family member, has been shown to be required for HSC survival (Opferman et al., 2005).

6. Quiescence or self-renewal

In order to both maintain a supply of mature blood cells and not exhaust HSCs throughout the lifetime of an individual, under steady state, most HSCs remain quiescent and only a small number enter the cell cycle. However, in response to hematopoietic stress such as blood loss, HSCs exit quiescence and rapidly expand and differentiate to repopulate the peripheral hematopoietic compartments. When quiescence is disrupted, HSCs displayed defective maintenance in G0 phase of cell cycle, leading to premature exhaustion of the stem cell pool under conditions of hematopoietic stress, impaired self-renewal, and loss of competitive repopulating capacity, eventually causing hematological failure.

Quiescence of HSCs is not only critical for protecting the stem cell compartment and sustaining stem cell pools over long periods, but it is also critical for protecting stem cells by minimizing their accumulation of replication-associated mutations. The balance between quiescence and proliferation is tightly controlled by both HSC-intrinsic and -extrinsic mechanisms. Understanding quiescence regulation in HSC is of great importance not only for understanding the physiological foundation of HSCs, but also for understanding the pathophysiological origins of many related disorders.

In steady state conditions HSCs are in a slowly dividing state, termed relative quiescence, with a cell division cycle in the mouse in the range of 2–4 weeks, localized in close contact with stromal cells, including osteoblasts (Calvi et al., 2003; Zhang et al., 2003). This is in contrast to the rapidly cycling hematopoietic progenitor cells, which are more committed to differentiation than HSCs. The balance between quiescent and cycling stem cells was proposed to rely on the amount of soluble cytokines, which result in HSCs relocating from the osteoblastic to the vascular niche (Heissig et al., 2002). However new results indicate that it depends on a complex network of signals.

Fig. 1. Networks interaction model for: A) quiescence, B) self-renewal and C) survival.
In part, the dramatic contrast in cell cycle status between stem and progenitor cells has led to the hypothesis that cell cycle regulation plays a fundamentally important role in stem cell fate determination. This hypothesis is supported by recent data demonstrating a slower rate of division in Hoxb4hiPbx1lo cells, which extensively self-renewal in vitro, compared to control cells (Cellot et al., 2007). It is essential for an HSC to undergo cell division if it is to self-renew, but how the cell division cycle is integrated into the process of self-renewal is unclear. It is also unknown as to whether cell cycle regulation represents an intrinsic or extrinsic modifier of HSC fate.

6.1 How HSC maintain quiescence

Negative regulators of both Cdk2 and Cdk4/6 activity, and therefore Rb function, have been demonstrated to have roles in regulating HSCs (Cheng et al., 2000; Janzen et al., 2006; Stepanova & Sorrentino, 2005; Van Os et al., 2007; Walkley et al., 2005). For the most part however these phenotypes have been relatively subtle, particularly when compared to hematopoietic phenotypes apparent after disruption of transcription factors such as C/EBPa (Hock et al., 2004) and Tel (Zhang et al., 2004) amongst others, and are often apparent only after serial transplantation. The "Rb pathway" has also been implicated in phenotypes observed in both the Bmi1-/- and ATM-/- HSCs (Ito et al., 2004; Lessard & Sauvageau, 2003; Park et al., 2003). The interaction of cell cycle regulators with other factors such as Hoxb4 or telomerase deficiency has produced much more striking phenotypes than that observed for the cell cycle mutants in isolation (Choudhury et al., 2007; Miyake et al., 2006). While clearly demonstrating that cell cycle modifiers have roles in regulating stem cells, particularly HSCs, the aforementioned studies have not been able to clearly discriminate between intrinsic or extrinsic contributions to HSC fate as all studies to date had utilized non-hematopoietic restricted mutant alleles. A study demonstrating that the p27Kip1-/- microenvironment mediates the myelo-lymphoid expansion observed in the p27Kip1-/- animals raises the possibility that the HSC expansion observed in p27Kip1-/- bone marrow is extrinsic in nature (Chien et al., 2006; Walkley et al, 2005). This result suggested that cell cycle regulators might play a role in regulating the competence of the hematopoietic niche, in addition to having potential intrinsic roles in HSC fate determination. Moreover Daria et al (2007) observed a requirement for Rb in the stress response of HSCs and this has also previously been suggested in the context of the role of Rb in erythropoiesis (Spike et al., 2004; Spike & Macleod, 2005).

Also of note is that the cell division dynamics of HSCs change during development, from rapidly cycling and dividing cells during the fetal liver and early stages of life to relatively quiescent and more slowly cycling in the adult context (Bowie et al., 2007; Bradford et al., 1997; Ito et al, 2000; Kiel et al., 2007; Sato et al., 1999). Thus the role for Rb may be context dependent, both in terms of stress response and developmentally in the regulation of HSC fate.

One important point that is becoming clearer recently is how some HSCs are maintained quiescent while others enter self-renewal program. Although bone–lining cells in the endosteal surface are often described as osteoblasts in the literature, they are heterogeneous in their degree of differentiation, and only a minority of these cells are actually bone synthesizing osteoblasts. So a good hypothesis is that in the endosteal niche some cells are in contact with true osteoblasts that expresses the necessary factors to maintain quiescence while others are not receiving the same signalization so will follow other fate.
Li (2008) proposed that HSC quiescence is maintained through several signaling pathways including positive and negative regulators from extrinsic and intrinsic factors already described. In this context the Tie-2/Ang-1 signaling pathway plays a critical role in the maintenance of HSCs in a quiescent state in the bone marrow niche (Adams et al., 2006). HSCs express the receptor tyrosine kinase Tie-2 and osteoblasts are the source of the Ang-1 ligand for Tie-2. Tie-2/Ang-1 signaling activates its key downstream targets, \( \beta_1 \)-integrin and N-cadherin in lineage-negative, Sca-1, C-kit double-positive (LSK), and Tie-2-positive cells, and promotes HSC interactions with extracellular matrix and cellular components of the niche. This interaction is sufficient to maintain the quiescence and enhanced survival of HSCs by preventing cell division (Arari et al., 2007). Ang-1/Tie-2 signaling also activates the phosphatidylinositol 3-kinase/Akt signaling pathway (Visnjic et al., 2004). Phosphatidylinositol 3-kinase/Akt signaling regulates several cell-cycle regulators, such as the CDK inhibitor, p21, which in turn leads to HSC quiescence.

Other pathway that has been proven to enhance quiescence is TPO/MPL. TPO is secreted by osteoblast while MPL is expressed in the membrane of HSCs. Interaction of these two proteins maintain HSCs attached to osteoblasts by activation of a pathway that results in the expression of their adhesion molecules targets and at the same time activate genetic programs which will control entry in cell cycle and survival of HSC. How these positive regulators interact with other positive and negative regulators is not completely understood. Moreover, which signaling pathways are being activated and which genes have their expression changed awaits to be clarified.

A recent study by Wang et al. (2009) recently identified STAT5, a downstream target of MPL, as a positive regulator of HSC quiescence by analyzing \( \text{STAT5}^{-/-} \) mice. Expression of quiescence regulators including Tie-2 and p57 are decreased in \( \text{STAT5}^{-/-} \) HSCs. This study demonstrated that \( \text{STAT5} \) might mediate MPL effects in maintaining HSC quiescence during steady state hematopoiesis and that the same pathway directly or indirectly regulates Tie-2 and p57. Interestingly, up-regulation of p57 is essential for TGF-\( \beta \)-induced cell-cycle arrest. How these pathways are connected awaits more investigation.

Two other signaling pathways that act as positive and negative regulators of quiescence deserve more discussion, the Hypoxia induced factor (HIF) and Osteopontina signaling pathway. Hypoxia microenvironment seems to be important for maintaining HSC quiescence The molecular mechanisms for this involve the hypoxia-inducible factor-1a (HIF-1a) regulated gene expressions in stromal cells. Two genes known to be targets of the HIF pathway are c-Kit and stromal cell derived factor-1 (SDF-1 or CXCL12) that both have proven to be important to HSC maintenance.

Osteopontina (OPN) is a negative regulator of HSC quiescence as an OPN-null microenvironment is sufficient to increase the number of stem cells associated with increased stromal Jagged-1 and Ang-1 expression and reduced primitive hematopoietic cell apoptosis. OPN seems to function by preventing HSC cycling. It is interesting to note that the release of this inhibition occurs in parallel with the possible activation of the Notch pathway.

As we can see there is many connections between intrinsic factors and extrinsic cues and between different intrinsic factors or different extrinsic factors. Some intrinsic factors function through affecting extrinsic factors, such as c-Myc, which negatively regulates HSC
quiescence by controlling $N$-cadherin expression level, reducing the $N$-cadherin–mediated interaction between HSCs and niche. Some extrinsic cues function through certain intrinsic factors, such as STAT5, which may serve as a component of MPL–induced signaling pathway, mediating MPL’s effects in maintaining HSC quiescence.

Interestingly, among those HSC quiescence regulators that have been identified so far, the majority are positive regulators; few are negative for the maintenance of HSCs quiescence. This is consistent with the idea showing that the bias toward reduced gene expression that actively maintains HSC quiescence is an important mechanism of HSC proliferation, suggesting that various positive regulators of HSC quiescence are actively restricting proliferation of HSCs, and that there may exist signals in the environment to promote HSC proliferation.

6.2 Deciding for self-renewal

Many signaling pathways are thought to contribute to stem cell self-renewal in the marrow niche including Notch (Maillard et al., 2003), Wnt (Duncan et al. 2005; Reya et al., 2003; Willert et al., 2003) and Hedgehog (Baron, 2001; Bhardwaj et al., 2001; Gering & Patient 2005).

Activated Notch expands the stem and progenitor cell compartment by either influencing undifferentiated cells to adopt a HSC fate or by causing a Go HSC population to up-regulate runx1-dependent gene expression. Findings that the stem cell markers runx1, sel, and lmo2 were transcriptionally increased in response to NICD (Notch Intra-Cytoplamic Domain) indicate that stem and progenitor cells were expanded in the adult marrow, possibly by increasing stem cell self-renewal. A conditional allele of runx1 was generated in the mouse to study the loss of Runx1 function during adult hematopoiesis (Growney et al., 2005; Ichikawa et al., 2004). In transplantation studies, Runx1-excised marrow cells showed a reduced competitive repopulating ability in long-term engraftment assays (Growney et al., 2005), demonstrating that Runx1 is essential for normal stem cell function.

The Wnt/β-catenin signaling pathway also plays a crucial role during self-renewal of HSCs (Nemeth & Bodine, 2007). Deregulation of this pathway has been implicated in the formation of solid tumors, like lung epidermal adenocarcinomas, breast carcinomas and intestinal colorectal tumors just to mention a few (Reya & Clevers, 2005). Although several Wnt genes are expressed in bone marrow, the precise role of Wnt signaling pathway in HSCs and its mechanism(s) of action remained unclear until very recently.

There is a multitude of Wnt signaling cascades some of them regulating one another. Using different receptors, Wnt proteins can trigger at least three intracellular signaling pathways: the canonical β-catenin pathway, the non-canonical calcium pathway and the c-Jun N-terminal kinase pathway (Zeng et al., 2004). Several components of the Wnt signaling machinery have been shown to play a role in HSC self-renewal. Both canonical as well as non-canonical pathways seem to be involved, since the canonical ligand Wnt3a intrinsically promotes self-renewal (Luis et al., 2009). On the other hand, the non-canonical ligand Wnt5a has been shown to extrinsically promote self-renewal by inhibiting canonical signaling (Murdoch et al., 2003). The mechanistic basis for the balance between canonical and non-canonical pathways is not fully understood. It is likely that numerous Wnt inhibitors or antagonists are modulating Wnt signaling.
Taken together, the existing studies suggest that canonical Wnt signaling may not be strictly required for HSC function, but that canonical Wnt signaling may affect self-renewal and differentiation of HSCs depending on the extent of canonical Wnt signaling and on the context of expression of additional genes. Non-canonical Wnt signaling and/or other signaling pathways may also compensate for the absence of canonical Wnt signaling in maintaining the self-renewal of HSCs (Huang, 2007).

To exactly control the fine tune of Wnt in HSC it is likely that the numerous Wnt-signaling inhibitors (Dickkopf homolog (Dkk), Wnt inhibitory factor (Wif) or secreted frizzled-related protein (Sfrp), or other Wnt antagonists, such as Kremen, Ctgf, Cyr61, Sost and Sostdc1) have to be the correctly expressed. Interestingly, some of these molecules also directly stimulate certain Fzds independent of Wnt factors. For example, Sfrp1 directly activates Fzd2, as well as Fzd4, and Fzd7 but can also interact with Wnt5a (Rodriguez et al., 2005; Dufourc et al, 2008; Matsuyama et al, 2009 & Kirstetter et al., 2006). This balance and feedback mechanisms between canonical and non-canonical Wnt signaling, suggests that \( \beta\)-catenin is the primary regulatory target of Wnt signaling. However, overexpression or stabilization of \( \beta\)-catenin results in expansion of the HSC pool, but, at the same time, the loss of myelopoiesis is due to a differentiation block (Renstrom et al., 2010), suggesting that \( \beta\)-catenin promotes self-renewal and/or inhibits differentiation.

Conversely, Wnt signaling also induces increased expression of HOXB4, Bmi1 and targets of Notch-1, genes that are implicated in self-renewal of HSCs. Transcription factors of homeodomain family (HOX family) have been found to regulate HSC self-renewal and downregulate differentiation. Disruption of HOX genes in mice led to abnormalities in multiple hematopoietic cell lineages. Moreover, overexpression of HOX genes (like HOXB4) has been associated with HSCs ex vivo expansion and HOX gene mutation with acute leukemia. Bmi1, a polycomb gene, seems to have a repressor role over p16 inhibiting apoptosis of HSCs and thus contributing to its maintenance. So the correct Wnt signaling seems to be essential to integrate the intracellular response in the decision to self-renew or differentiate (Reya et al., 2003).

The investigation of the interactions between Bmi1 and Hoxb4, showed that Bmi1 is not required for the in vivo expansion of fetal HSCs but is essential for the long-term maintenance of adult HSCs. Moreover, Hoxb4 overexpression induces an expansion of Bmi1-/- STR-HSCs leading to a rescue of their repopulation defect. Together, these results support the emerging concept that fate and sustainability of this fate are two critical components of self-renewal in adult stem cells such as HSCs.

Moreover Polycomb group (PcG) proteins play a role in the transcriptional repression of genes through histone modifications. Recent studies have clearly demonstrated that PcG proteins are required for the maintenance of embryonic as well as a broad range of adult stem cells, including hematopoietic stem cells (HSCs). PcG proteins maintain the self-renewal capacity of HSCs by repressing tumor suppressor genes and keep differentiation programs poised for activation in HSCs by repressing a cohort of hematopoietic developmental regulator genes via bivalent chromatin domains. Enforced expression of one of the PcG genes, Bmi1, augments the self-renewal capacity of HSCs. PcG proteins also maintain redox homeostasis to prevent premature loss of HSCs. These findings established PcG proteins as essential regulators of HSCs and underscored epigenetics as a new field of HSC research (Li et al., 2010; Komuna, 2010).
Recently we demonstrated that another polycomb group member, Suz12 gene, is activated by the non canonical Wnt pathway and may epigenetically inhibit genes involved in hematopoietic differentiation. These data pointed to cell cycle changes, deregulation of early differentiation genes and regulation of PRC2 polycomb complex genes, due to Suz12 role in CML blast crisis. This observation indicates that the cross talk between Wnt and Polycomb pathways may promotes hematopoietic differentiation. (Pizzatti et al., 2010).

Taken together all these data fits in a model were HSCs fated to self renew are in contact in the endosteal niches with osteoblasts expressing Notch legands (Jagged) so the pathway that will be induced is Notch pathway.

The Hedgehog (Hh) is a ligand that binds and represses the Patched receptor and thereby releases the latent activity of the multipass membrane protein Smoothened, which is essential for transducing the Hh signal. Using Patched+/- mouse with increased Hh signaling activity, it was demonstrated that constitutive activation of the Hh signaling pathway results in the steady-state accumulation of phenotypically defined HSCs and an increase in the proportion of cycling cells within this population (Kuhn et al., 1995). However, HSC activity on secondary transplantation is reduced 3-fold, indicating the functional exhaustion of the HSC pool in this mutant. In vivo treatment with an inhibitor of the Hh pathway rescues these transcriptional and functional defects in HSCs. This study establishes Hh signaling as a negative regulator of the HSC quiescence. In contrast to the germline Patched+/- mode, the mode of conditional deletion of Smoothened in the adult hematopoietic compartment was used in other two studies. However, the negative effects of the Hh pathway on HSC quiescence were shown in one study (Walkley & Orkin, 2006) and not in another (Stead et al., 2002). The discrepancy is possibly due to a distinct mode of deletion. How this pathway collaborate with the two others is not clear although interaction through GSK3 have been already proposed.

The outcome of Hh signaling varies according to the receiving cell type. GLI, the cytoplasmic effector of Hh signaling activates the transcription of several target genes as CyclinD1 and D2,N-Myc,Wnts, FoxM1,Hes 1,Bcl2,Osteopontin and others. If these genes are activated in HSCs has not yet been defined but if they are, a clear interconnection between several important signaling pathways is visualized.

One important point when talking about self-renewal is how to prevent exhaustion of the HSC pool.

7. Role of telomerase in hematopoietic stem cell

Stem cells self renewal capacity is believed to be closely associated with tissue degeneration during aging. Studies of human genetic diseases and gene-targeted animal models have provided evidence that functional decline of telomeres and deregulation of cell cycle checkpoints contribute to the aging process of tissue stem cells. Telomere dysfunction can induce DNA damage response via key cell cycle checkpoints, leading to cellular senescence or apoptosis depending on the tissue type and developmental stage of a specific stem cell compartment (Ju Z et al., 2011).

Studies in hematopoietic stem cell (HSC) biology are often focused on “self-renewal” and differentiation. Implicit in the word self-renewal is that the two daughter cells generated by
a self-renewal division are identical to the parental cell. Strictly speaking, this is not possible because DNA is continuously damaged and repaired by DNA-repair mechanisms that are not 100% efficient (Lansdorp et al., 2005).

It is important to note that the efficiency of DNA repair varies greatly among different stem cell types. For example, embryonic stem cells are quite resistant to DNA damage and maintain the length of telomere repeats on serial passage, whereas HSCs are quite sensitive to DNA damage and less able to maintain telomere length. This idea has given rise to the notion that many aspects of normal aging could primarily reflect limitations in DNA repair and telomere-maintenance pathways in the (stem) cells of the soma (Lansdorp et al., 2005).

The loss of telomere repeats in adult hematopoietic cells (including purified “candidate” HSCs) relative to fetal hematopoietic cells also fits a model that postulates a finite and limited replicative potential of HSCs (Vaziri et al., 1994; Lansdorp et al., 1995; Lansdorp et al., 1997). How this collaborates with the model of LT-HSCs given rise to ST-HSCs has not still been addressed.

Eukaryotic chromosomes are capped by special structures called telomeres, which are guanine-rich, simple repeat sequences. Telomeres act to guarantee chromosome integrity by preventing illegitimate recombination, degradation, and end fusions (Blackburn et al., 1991; Stain et al., 2004).

Synthesis and maintenance of telomeric repeats are accomplished by a specialized ribonucleoprotein complex known as telomerase. Telomerase consists of an essential RNA template and protein components, one of which appears to resemble reverse transcriptase. In the absence of telomerase, the failure of DNA polymerase to fully synthesize DNA termini leads to chromosome shortening (Stain et al., 2004; Lee et al., 1998).

In contrast to mice were short telomeres maintain cell survival for some generations, a modest two fold reduction in telomerase levels in humans (resulting from haploinsufficiency for the telomerase RNA template gene) typically results in premature death from complications of aplastic anemia or immune deficiency. Recent studies indicate that short telomeres and eventual marrow failure may also result from haploinsufficiency for the telomerase reverse transcriptase (hTERT) gene (Yamaguchi et al., 2005).

Moreover the large number of HSCs typically used in clinical transplant settings may effectively prevent their replicative exhaustion. Variations in telomere length between cells and individuals have even made it difficult to reproducibly document a decline in telomere length following transplantation. Nevertheless, a significant shortening of telomeres was observed in the first year after allogeneic bone marrow transplantation (Landsdorp et al., 2005). Furthermore, marrow failure with pronounced telomere shortening has been described in a few long-term survivors of HSC transplants. Although there is little evidence to suggest that telomere shortening will result in an epidemic of marrow failure in HSC transplant recipients, caution remains warranted when the cell number available for transplantation is limited or when the telomere length in HSCs for transplantation is short, as in cells from old donors or patients with telomerase deficiencies. It is tempting to speculate that some of the advantages of cord blood HSC transplants are related to the longer telomeres in individual cord blood HSCs (Awaya et al., 2002).
It has been proposed that telomeres can switch between an open state (in principle allowing elongation by telomerase) and a closed state (inaccessible to telomerase) with the likelihood of the open state inversely related to the length of the repeat track (Blackburn et al., 2001). In most human cells, telomerase appears to be present at limiting levels, allowing elongation of only a limited number of critically short telomeres. Accumulation of short telomeres before replicative senescence has been observed and replicative senescence or apoptosis could result when the number of critically short telomeres exceeds the telomere repair capacity in a cell (Ju Z et al., 2011). In this context the ability of HSCs to modulate telomerase activity may be crucial in maintaining the self-renewal process. In human BM cells, low telomerase activity levels were demonstrated in multipotent HSCs, whereas significant upregulation of enzyme activity was apparent in the presence of proliferation-inducing cytokines (Samper et al., 2002; Stein et al., 2004). So cytokines and JAK-STAT signaling pathway may contribute to self renewal by maintaining telomerase activity.

The role of DNA repair pathways and telomeres/telomerase in the biology of normal and malignant human HSCs cells as well as the biology of aging clearly needs further study. New insight in the role of telomerase in HSCs has been provided by recent studies of patients with inborn errors in telomerase activity. Therefore, a further understanding of the molecular mechanisms underlying HSC aging may help identity new therapeutic targets for stem cell-based regenerative medicine.

8. Asymmetric cell division – A mechanism to generate progenitors while maintaining HSC

In both invertebrates like the insect Drosophila, and mammals, the major characteristic of stem cells is their ability to self-renew. Using various modes of proliferation, stem cells maintain or expand the available stem cell pool, but they can also generate more specialized progeny that constitute the majority of cells in an adult individual. In multi-cellular organisms, totipotent zygotes generate pluripotent stem cells, which become increasingly restricted in their lineage potential during development, and subsequently give rise to mature tissue-specific, multipotent stem cells. Stem cells show either ‘proliferative’ symmetric divisions or ‘differentiative’ asymmetric divisions to regulate a balance between the maintenance of stem cell pool and the supply of mature cells. It is critical for stem cells to tightly control this balance between the two different modes of division, both during development and adulthood, because, failure in maintaining cellular homeostasis may lead to incomplete tissue or organ development, whereas uncontrolled proliferation can lead to tumorigenesis.

Symmetric cell divisions commonly occur during development of invertebrates and vertebrates, phenomena that can also be observed during wound healing and regeneration of tissues. This mode of division is defined by the generation of two daughter cells that acquire the same fate, thereby expanding the pool of stem cells required or generating two differentiating daughter cells. Asymmetric cell divisions play a key role in generating cellular diversity during development by generating two daughter cells that are committed to different fates in a single division, simultaneously self-renewing to generate a daughter cell with stem cell properties, as well as to give rise to a more differentiated progeny.
Asymmetric stem cell divisions can be controlled by intrinsic mechanisms or the asymmetric exposure to extrinsic cues. Intrinsic mechanisms use apical-basal or planar polarity along the mitotic spindle to asymmetrically segregate cell fate determinants into only one daughter cell. Extrinsic mechanisms rely on contact with the so-called stem cell niche, a cellular microenvironment that provides external cues (Doe, 2008; Li & Xie, 2005; Morrison & Spradling, 2008). Orientation of its mitotic spindle perpendicular to the niche surface allows the asymmetric segregation of cell fate determinants relative to the external stimuli to maintain self-renewal potential.

Much progress has been made in understanding intercellular mechanisms, especially the identification of niches for various types of tissue stem cells and elucidation of the role of the niche in regulating asymmetric stem cell division.

Although the role of niche in the asymmetric division of mammalian stem cells has not been clearly illustrated, Fuchs (2008) have shown that embryonic basal epidermal cells use their polarity to divide asymmetrically with respect to the underlying basal lamina, generating a committed suprabasal cell and a proliferative basal cell. Because skin stem cells are a subpopulation of mitotically active basal epidermal cells, it is conceivable that these stem cells divide in an asymmetric fashion to self-renew and to produce differentiated keratinocytes. Moreover, integrins and cadherins in the basal lamina are essential for the proper localization of apical complexes containing atypical PKC (aPKC), the Par3 – LGN – Inscuteable protein, and NuMA (nuclear mitotic apparatus protein) – dynactin. This asymmetric localization may be functionally important because similar complexes in Drosophila neuroblasts are essential for asymmetric division (Chia et al., 2008).

In addition to basal epidermal cells, mouse neuroepithelial stem cells and hematopoietic precursor cells undergo both asymmetric and symmetric divisions. In the mammalian central nervous system, embryonic neuroepithelial cells first undergo symmetric division to expand their population and then switch to asymmetric divisions for neurogenesis. This switch involves a change in cleavage plane orientation from perpendicular to parallel to the plane of the apical lamina, leading to an asymmetric distribution to the daughter cells of the apical plasma membrane, which constitutes only a minute fraction (1 – 2%) of the entire neuroepithelial cell plasma membrane (Kosodo et al., 2004). Somewhat similarly, mouse hematopoietic progenitor cells are capable of both symmetric and asymmetric divisions in cultures supported by stromal cells (Wu et al., 2007). A pro-differentiation stromal cell line increased the frequency of asymmetric division, whereas a pro-proliferation stromal cell line promoted symmetric division. These observations indicate that niche signaling can also control the asymmetry of stem cell division at a populational level.

Although niche induction accounts for asymmetric division in some types of stem cells, it may not play a role in all types of stem cells. In some stem cells there is an intrinsic polarity where molecules are segregated along an axis and serves as determinant of cell fate after cell division. In these cases the orientation of mitotic spindle has to be coordinated with the asymmetric localization of the cell fate regulators. This is the case, for example of Numb homologue during hematopoietic precursor cell division. So looking for hematopoietic niche and signaling between microenvironment cells and the stem cell we could “envisage” (imagine; idealize, construct) a model for stem cell to decide between symmetric or asymmetric division.
A particularly exciting development in basic stem cell research in the past few years is the discovery of novel functions of cell cycle regulators in controlling the asymmetry of stem cell division, as timely reviewed by Chia et al (2008). For example, the cdc2/cdk1 level controls whether a neural or muscle progenitor undergoes symmetric or asymmetric division. In neuroblasts, high levels of CDK1 during mitosis are required for the asymmetric localization of apical and basal protein complexes. In addition, Aurora and Polo kinases act as tumor suppressors in neuroblasts by preventing excess self-renewal, implicating the function of asymmetric division in restricting over-proliferation. The mutations of these two kinase genes affect the asymmetric localization of aPKC, Numb, Partner of Numb, and Notch, causing symmetric division to generate two daughter neuroblasts. In addition, anaphase-promoting complex/cyclosome is also required for the localization of Miranda and its cargo proteins (Prospero, Brain Tumor, and Staufen). More surprisingly, even cyclin E, a G1 cyclin, is involved in asymmetric neuroblast division.

Interestingly in epidermal progenitors the decision of choosing between symmetric cell division or asymmetric cell decision is tightly regulated. Two control points have been indentified: expression of Inscutable and recruitment of NuMA to the apical cell cortex. Moreover in embryonic lung distal epithelium Eya1 protein regulates cell polarity, spindle orientation and the localization of Numb, which inhibits Notch signaling with the participation of NuMa protein (El-Hashad et al , 2011).

9. Conclusion

In the last ten years a body of evidence has accumulated on the hematopoietic stem cell niches and the mechanisms by which they regulate HSCs homeostasis. However many questions remain to be addressed. What we can speculate with today data is that in the endosteal niche dissimilar stages of differentiating osteoblasts provides diverse signals. These signals induce a quiescent or a self-renewal fate. However after leaving quiescence HSCs are prone to accumulate mutations that will lead to senescence. To prevent HSCs exhaustion mechanisms to enhance survival are also induced. Several of these additional signals come from the osteoblasts but also from other cells from microenvironment as stromal cells and endothelial cells. This is specially visualized in the decision of a symmetric or asymmetric division. From the control of all these interconnected pathways depends a normal hematopoiesis.

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11. References


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This book provides a comprehensive overview in our understanding of the biology and therapeutic potential of hematopoietic stem cells, and is aimed at those engaged in stem cell research: undergraduate and postgraduate science students, investigators and clinicians. Starting from fundamental principles in hematopoiesis, Advances in Hematopoietic Stem Cell Research assemble a wealth of information relevant to central mechanisms that may regulate differentiation, and expansion of hematopoietic stem cells in normal conditions and during disease.

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