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Hematopoietic Stem Cell: Self-renewal versus Differentiation

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Abstract

The mammalian blood system, containing more than ten distinct mature cell types, stands on one specific cell type, hematopoietic stem cell (HSC). Within the system, only HSC possess the ability of both multi-potency and self-renewal. Multi-potency is the ability to differentiate into all functional blood cells. Self-renewal is the ability to give rise to HSC itself without differentiation. Since mature blood cells are predominantly short lived, HSC continuously provide more differentiated progenitors while properly maintaining the HSC pool size properly throughout life by precisely balancing self-renewal and differentiation. Thus, understanding the mechanisms of self-renewal and differentiation of HSC has been a central issue. In this review, we focus on the hierarchical structure of the hematopoietic system, the current understanding of microenvironment and molecular cues regulating self-renewal and differentiation of adult HSC, and the currently emerging systems approaches to understand HSC biology.

Introduction

While mature blood cells are produced at a rate of more than one million cells per second in the adult human [1], most of the hematopoietic stem cells (HSCs) from which they are derived cycle very infrequently and primarily reside in the G₀ phase of the cell cycle under homeostatic conditions [2]. These two facts present an interesting conundrum: how does the organism achieve a balance whereby an adequate pool of HSCs is maintained for the life of the organism, while at the same time HSCs consistently meet the organism's enormous demand for continuous replenishment of mature blood cells, most of which are short-lived. The importance of this balance is underscored by the numerous examples where aberrant HSC development causes severe disease *e.g.* when HSC differentiation into committed progenitors is not accompanied by the typical loss of self-renewal capacity, or HSC derived progenitors fail to fully differentiate into mature blood cells [3], and may enter a preleukemic progression [4]. These intriguing features of mammalian hematopoiesis have fueled extensive investigation of the system over the last several decades. In this review, we focus on the outlined conundrum, and discuss what is currently known of the regulatory events that govern the ability of HSCs to generate many billions of mature blood cells while at the same time maintaining an adequate pool of HSCs for the entire life of the species.

The Concept of Stem Cells

The “stem cell” concept was first proposed by Till and McCulloch following their pioneering studies of the blood system regeneration *in vivo*. Ten days after transplanting limiting number of syngenic bone marrow (BM) cells into recipient mice, they observed cellular colonies that formed in the spleens of recipient mice. Analysis of these colonies revealed that a very small sub-population of the donor BM cells possessed two remarkable

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properties: (1) the ability to generate multiple types of myeloerythroid cells, and (2) the ability to self-replicate [5–8]¹. These findings introduced the two defining criteria of stem cells *i.e.* multi-potency and self-renewal. Hematopoietic Stem Cells (HSCs) are the only cells within the hematopoietic system that possess the potential for both multi-potency and self-renewal. In the case of HSC, multi-potency is the ability to differentiate into all functional blood cells, while self-renewal is the ability to give rise to identical daughter HSCs without differentiation.

The field of stem cell research has greatly expanded since the initial studies of Till and McCulloch and now includes stem cells that give rise to specific organs/tissues (collectively termed tissue-specific stem cells) and also embryonic stem (ES) cells which can give rise to every cell type in the adult body. A system of nomenclature has evolved to reflect the differentiation potential of different stem cell populations (summarized in Table 1). It is beyond the scope of this article to discuss non-hematopoietic stem cell populations; excellent reviews of the latter cells are presented elsewhere in this issue.

In 1988, the initial prospective purification of hematopoietic stem cells from mouse BM was achieved utilizing the relatively new technologies of multi-color fluorescence-activated cell sorting and monoclonal antibodies [10,11]. The resultant population of enriched mouse HSCs had a surface marker phenotype of Thy-1^{low} Lin (Lineage-markers)⁻ Sca-1⁺, and represented approximately 0.05% of the mouse adult BM cells. Spangrude and colleagues demonstrated that these were the only cells in mouse BM capable of transferring long-term reconstitution of the entire hematopoietic system (then defined as more than 3 months) when transplanted into lethally irradiated mice [11]. A reductionist approach by Uchida et al showed that Thy1.1^{low}, but not Thy1.1^{high} or Thy1.1⁻ cells could give rise to donor-derived long-term multilineage reconstitution of irradiated hosts; this was true of Sca-1⁺, but not Sca-1⁻ cells, and of Lin⁻, but not Lin⁺ cells [12]. Since these initial studies, mouse HSCs have been more extensively purified by identifying and then utilizing additional cell-surface markers to distinguish them from other cells in BM; these included, but were not exclusively single cells that could self-renew and give long-term multilineage maturation [12–14]. In 1994 the population isolated by Spangrude et al was shown to include at least 3 multipotent populations: Long-Term (LT)-HSC, Short-Term (ST)-HSC, and Multi-Potent Progenitor (MPP, a cell population that has lost the self-renewal capacity of HSC) [15]. In 1996, HSCs from adult mouse BM were sufficiently enriched to conduct single-cell transplantation experiments, and these studies revealed that one in three CD34^{-/low} c-Kit⁺ Sca-1⁺ Lin⁻ cells showed myelolymphoid long-term reconstitution in lethally irradiated recipients after a single cell transplant [16]. Despite the fact that hematopoietic tissues contain both stem and progenitor cells, rapid and sustained engraftment of syngenic and even of H2 incompatible allogenic hosts can only be achieved with HSC, the time to engraftment depending on the number of HSC transplanted [17].

Hierarchical Structure of Mouse Hematopoietic System

The mammalian blood system contains more than ten distinct mature cell types including red blood cells (erythrocyte), megakaryocytes/platelets, myeloid cells (monocyte/macrophage and granulocytes), mast cells, T- and B- lymphocytes, natural killer (NK) cells and dendritic cells (DCs). The concept that such diverse cell types are all derived from a common progenitor cell, *i.e.* from HSCs, is intriguing and an indication that HSCs possess

¹Subsequent studies have shown that the day 10 spleen colonies are derived from committed, multipotent, myeloerythroid progenitors 9. Nakorn, T.N., T. Miyamoto, and I.L. Weissman. *Characterization of mouse clonogenic megakaryocyte progenitors*. Proc Natl Acad Sci U S A, 2003. **100**(1): p. 205-10. This does not detract from the conceptual origins of the field of stem cell biology by the Till-McCulloch group.

remarkable differentiation potential. To understand how the multi-potent HSC differentiate into these diverse functional cell types, our laboratory and others have used cell surface marker phenotype analysis by flow-cytometry to identify and isolate discrete sub-populations of developing blood cells, together with a battery of well-defined and highly sensitive read-out assays to elucidate their relative functions and differentiation potential. These analyses have suggested a hierarchical structure in hematopoietic development in which multi-potency is progressively restricted (illustrated diagrammatically in Figure 1). HSCs initially give rise to the MPPs which no longer possess self-renewal ability yet keeping full-lineage differentiation potential [15,18]. We now realize that this population of MPPs is still heterogeneous [16,18,19] and additional work to fully understand this heterogeneity is in progress [20–22]. Further downstream, MPPs advance to oligopotent progenitors: 1) the common lymphoid progenitor (CLP) [23–25] and 2) the common myeloid progenitor (CMP) [26]. Collectively these oligopotent progenitors then give rise to all the lineage-committed effector cells of the hematopoietic system *e.g.* CMPs give rise to megakaryocyte/erythrocyte progenitors (MEPs) and granulocyte/macrophage progenitors (GMPs) [9,27]. The position of DCs in this model is interesting. We have shown that the three major phenotypic subsets of these antigen-presenting cells ($CD8\alpha^+$ DC, $CD8\alpha^-$ DC, and plasmacytoid DC) can be derived either from CMP and CLP [28–30]; the biological functions of the 6 different DC subsets identified in our studies are yet to be elucidated.

Human HSC and Downstream Progenitors

Human HSCs were isolated using similar technologies to those used for mouse HSCs, *i.e.* isolation of cells representing different stages of differentiation on the basis of cell-surface marker phenotype, coupled with functional assays. For human hematopoiesis, the property of long-term reconstitution of the various cell subsets is evaluated in xenotransplantation models, utilizing immuno-deficient mice, sometimes transplanted with fetal human hemolymphoid organs for irradiation-reconstitution assays [31,32]. The first cell-surface marker used to enrich human HSCs was CD34, a ligand for L-selectin that is expressed by only 0.5–5% of blood cells in human fetal liver, cord blood and adult BM [33–35]. *In vitro* assays revealed that almost all $CD34^+$ cells have multi-potency or oligo-potency, but also that the population was still very heterogeneous.

The first prospective isolation of human HSC exhibited the phenotype of $CD34^+CD90^+Lin^-$, where the Lin markers include T, B, NK, and Myeloerythroid specific markers [31]. These cells generated lymphoid and myeloid progeny in both *in vitro* colony assays and *in vivo* SCID (severe combined immunodeficiency)-hu mice; in contrast, the remaining population of $CD34^+CD90^-Lin^-$ cells were unable to generate cell clones containing myeloid and lymphoid cell types [31]. Further enrichment of human HSCs within the $CD34^+$ population was achieved via differential expression of the surface marker CD38. Although most of $CD34^+$ cells (90–99%) co-express CD38, cells that can give rise to multi-lineage colonies containing both lymphoid and myeloid cells reside in the CD38 low to negative fraction and the $CD90^+$ fraction. [31,36–39]. Furthermore, $CD34^+CD38^-$ cells and not $CD34^+CD38^+$ cells are highly enriched for long-term culture-initiating cells [36,40] and contain cells capable of repopulating the absent lymphoid compartment of SCID mice [31] and NOD (non-obese diabetes)-SCID immunodeficient mice [41,42]. Indeed enrichment of human HSCs from G-CSF mobilized peripheral blood on the basis of CD34 and CD90 co-expression has improved the efficiency of human bone marrow transplantation by providing cells that mediated hematopoietic reconstitution and long-term engraftment but which have greatly reduced contamination of unwanted host cancer cells or immunoreactive T cells [43–45]. Virtually all $CD34^+CD90^+Lin^-$ cells reside in the $CD38^-$ fraction [39], leading to the overall conclusion that human HSC are enriched in the $Lin^-CD34^+CD38^-CD90^+$ population of human sources of hematopoietic cells.

While this latter population of Lin⁻ CD34⁺ CD38⁻ CD90⁺ human cells is greatly enriched for human HSCs, retroviral or lentiviral marking of this population prior to xenotransplantation into immunodeficient mice has revealed functional heterogeneity of the cells [46–48]. Our own studies have recently identified a human MPP population in human cord blood defined as Lin⁻ CD34⁺ CD38⁻ CD90⁻ CD45RA⁻ which exhibit the functional properties of multipotency and an incomplete self-renewal capacity [49]. Further downstream of MPP, early myeloid and lymphoid committed progenitors have been identified in the human hematopoietic system that give rise to likely counterparts of murine CMP, GMP, MEP, and CLP [1,50–53]. Using IL-3Ra and CD45RA (an isoform of CD45 that negatively regulates some select classes of cytokine signaling), human CMP, GMP, and MEP populations have been identified and verified through *in vitro* and *in vivo* assays [53].

In both mouse and human hematopoiesis, this complex multi-tiered scheme (see Figure 1) allows for an enormous amplification in the numbers of terminally differentiated cells, while at the same time maintaining precise regulation of stem cell homeostasis

Niche: Essential Microenvironment for HSC

Essential insights into molecular mechanisms regulating HSC self-renewal and differentiation were provided prior to HSC isolation. Investigations of two mast cell-deficient spontaneous mutant mouse strains, the W strain [54], and the S1 strain [55], lead to the concept of a ‘niche’ *i.e.* an essential microenvironment for HSCs. The W gene locus is on chromosome 5, and the S1 gene locus is on chromosome 10. Mutant mice of both W gene and S1 genes show quite similar phenotypes; anemia, lack of mast cells, lack of melanocytes, and lack of germ cells [56–62]. When normal BM cells are transplanted into W mutant mice, the hematopoietic system is completely restored. However, when transplanted into the S1 mutant mice, hematopoiesis is still abnormal. When BM cells from the W mutant mice are transplanted into either wild-type or the S1 mutant mice, hematopoiesis is not corrected. On the other hand, when BM cells of the S1 mutant mice are transplanted into the W mutant mice, hematopoiesis is reconstituted. These observations lead to the proposal of a model that a specific microenvironment or ‘niche’ comprising cells outside the hematopoietic system is required for self-renewal and differentiation of HSCs. The transplantation experiments just outlined were best explained by concluding that the gene on the W locus is essential for functional HSCs and the gene on the S1 locus is essential for some environmental element essential for hematopoiesis but not contained in bone marrow hematopoietic cells *i.e.* so-called “niche” [63–66]. In 1990, the critical gene in the W locus was found to be that encoding cytokine receptor-tyrosine kinase c-Kit [67,68]; at approximately the same time, the important gene in the S1 locus was found to encode steel factor (SLF), also known as kit-ligand or Stem Cell Factor (SCF) [69]. The bone marrow niche for HSC in mice has been partially characterized: Clonal bone marrow stromal cells have been found that support HSC self-renewal and myelo-lymphoid maturation [70]. Phenotypic populations enriched for HSC have been located near the endosteum [71,72] and near blood vessels in the bone marrow [19]. The bone structures that include bone and HSC niches can be reconstituted ectopically with highly purified fetal bone osteochondral stem/progenitor cells, but not osteogenic progenitors alone [21]. These findings are evidence that the interactions between the receptors and ligands between HSCs and the niche are essential for maintenance of the hematopoietic system.

Molecular cues governing HSC

Although many cytokines and their receptors have been investigated utilizing gene-targeting technology, few receptors/ligands have been shown to play essential roles in HSC function (Table 2, 3). Two such receptor/ligand pairs that are critical for HSC are SCF/c-Kit

(described above), and TPO/c-Mpl. Studies using prospectively isolated mouse HSC have shown that both c-Kit and c-Mpl are expressed on highly purified HSC [13,73–78], and genetic elimination of either TPO or c-Mpl leads to reduction of HSC [75,76]. Consistent with these data, cytokines SCF and TPO can both support survival and proliferation of purified mouse HSC assayed in serum-free culture at the single cell level [79]. TPO signaling of HSC is thought to occur via an association ligand-bound c-Mpl with the adaptor protein Lnk, an intracellular scaffold protein that acts as an inhibitor of many cytokine signaling pathways, including SCF, EPO, IL-3, IL-7 as well as TPO [74,79–82]. Detailed studies of Lnk^{-/-} mice have revealed not only increased absolute numbers of HSC but also increased repopulation potentials of individual HSC in competitive repopulation assays, suggesting that Lnk is a negative regulator of HSC self-renewal [74,79,83]. Moreover, TPO, not SCF, was the responsible signaling pathway for the increased self-renewal capacity of Lnk^{-/-} HSCs [74,79], a finding consistent with earlier studies that the generation of fetal liver HSC is not impaired in Sl/Sl or W/W^v mice [13]. These studies suggested that TPO signaling is a positive regulator of HSC self-renewal, and Lnk is a key negative regulator of self-renewal signaling of HSC.

In addition to SCF and TPO, the availability of purified HSCs and recombinant proteins has enabled investigation of the direct effects of cytokines on HSC. Functional effects of many cytokines including IL-3, IL-6, IL-11, Flt-3 ligand in combinations with either SCF and/or TPO have been reported. In most cases, exposing HSCs to combinations of these cytokines results in survival and proliferation of cells. However, in most studies, these cells immediately lose long-term repopulation potential as assessed in transplantation assays. Later studies have reported that the Flt-3 receptor is not expressed on HSCs [18,73]. Similarly, the IL-11 receptor knockout mice showed normal hematopoiesis [84], question an essential role for this receptor-ligand system on HSC function. In terms of the latter apparent contraction, it has now become clear that many cytokines have redundant functions at the level of either receptor binding or intracellular signal transduction. Thus further investigation is required, with comprehensive study involving antagonism of multiple related receptors in order to clarify this complex area.

Apart from classical hematopoietic cytokines, developmental signaling pathways have also been shown to be relevant for adult HSC. Purified Wnt3a treatment *in vitro* increases self-renewal of mouse adult HSCs [85,86]. Overexpression of constitutively active β -catenin, an intracellular transducer of Wnt signaling, in mouse HSCs followed by long-term culture results in dramatic expansion of functional HSC as measured by both cell-surface phenotype and transplantation assays. Consistent with these observations, ectopic expression of axin or a frizzled ligand-binding domain, both mediators that inhibit the Wnt signaling pathway, lead to inhibition of HSC growth *in vitro* and reduced constitution activity after transplantation [86]. However, subsequent gain- and loss-of-function studies yielded more complicated results. Two independent studies utilizing *in vivo* conditional expression of β -catenin revealed impaired multilineage differentiation and a transient increase in HSC numbers, followed by exhaustion of the HSC pool [87,88]. Furthermore, opposite approaches to conditionally inactivation of β -catenin in HSCs showed contradictory results with some of these studies showing normal hematopoiesis and HSC activity in the combined absence of β -catenin and its homologue γ -catenin [89–92]. These discrepancies may reflect an elaborate redundancy of Wnt signaling molecules, the presence of compensatory signal transduction pathways, and the complicated dose-response of signal transduction, thus unresolved mechanisms behind the effects remain to be addressed. (For further review: <http://www.stanford.edu/~rnusse/wntwindow.html>)

In contrast to the high turnover of lineage-restricted progenitors, most of HSCs reside in the ‘quiescent’ G₀ phase of the cell cycle [2]. TGF- β /Smad signaling is one of the responsible

pathways that maintain quiescence of HSCs. *In vitro* culture studies have revealed inhibitory effect of TGF- β on HSC proliferation without inducing apoptosis [93–95]. Moreover, neutralization of TGF- β *in vitro* was shown to release early hematopoietic progenitor cells from quiescence [96–98]. Several molecular mechanisms have been proposed for TGF- β mediated inhibition of HSC proliferation, including alterations in cytokine receptor expression, inhibition of lipid raft clustering which amplifies cytokine signaling, and up-regulation of cyclin-dependent kinase inhibitors *e.g.* p21 and p57 [96,99–109]. However, loss-of-function strategies to elucidate direct effect of TGF signaling on HSC have been challenging. Despite various phenotypes in mature blood cells, conditional knockout mice of the TGF- β type I receptor displayed normal HSC self-renewal and regenerative activity *in vivo* [110], and mice deficient for the TGF- β type II receptor have not been well characterized in the context of HSC function. Furthermore, due to the high redundancy of the Smad molecules (which are the intracellular transducer of TGF signaling), and early embryonic lethality of most Smad and TGF- β knockout mouse models, *in vivo* elucidation of the role of the TGF/Smad signaling pathway in HSC function has proven difficult.

Another proposed cue for HSC quiescence is Ang-1/Tie2. Tie2 is a receptor tyrosine kinase expressed on endothelial cells and HSCs [111–113]. Despite the fact that Tie2 knockout mice models are embryonic lethal, studies utilizing chimeric mice containing normal, Tie1-null, or Tie2-null cells showed that these receptors are not required for prenatal hematopoiesis. However Tie2 is indispensable to maintain the HSC pool in adult BM [114]. Arai et al reported that in physiological conditions, HSCs expressing Tie2 are quiescent and reside next to BM osteoblasts which express Ang-1. Furthermore, *in vitro* Ang-1 treatment suppresses proliferation of HSCs and maintains *in vivo* long-term repopulating activity [115]. These results suggest that Ang-1/Tie2 signaling is an ideal target for modification of HSC quiescence.

Systems Approaches to Understand HSC Biology

The preceding sections summarize information about regulation of HSC fate that has been gained from traditional approaches utilizing gene overexpression, gene deletion, and direct stimulation of highly purified HSC with recombinant proteins. While valuable insights have been gained, these approaches are limited by the lack of demonstration of physiological *in vivo* relevance, including genetic redundancy underlying systems that are essential for host survival, such as the hematopoietic system. For example, a “back-up” gene or pathway can compensate functionally for the deleted gene in a gene knockout mouse in a manner that masks the true physiology of normal homeostasis. Our laboratory and numerous others have focused on a currently emerging alternative approach, termed systems approach, to shed light on the complex molecular network regulating HSC fate. Although the extremely low frequency of HSC in BM makes it difficult to obtain large numbers of pure HSCs, recent advances in microarray technologies have enabled systematic profiling of gene expression by small numbers of purified HSCs. Gene expression profiling studies of highly purified mouse adult HSCs are summarized in Table 4. Microarray analysis detects the concentration of target mRNAs encoded by genes expressed by a cell population by the high stringency hybridization of an mRNA preparation from the cell population to a microarray matrix expressing very large numbers of synthetic oligonucleotide probes. The efficiency of this event depends on target and probe sequences, and so is not uniform for each target mRNA. Based on this limitation, microarray assay provides only differentially expressed genes after comparison of more than 2 samples. When a particular cell population *e.g.* HSCs is compared to two other different cell populations, the list of differentially expressed genes between each cell population pairing will be different. Thus, interpretation of microarray studies depends on both method of the HSC purification, and also the counterpart cell population to which HSCs are being compared.

In 2002, Park et al generated microarrays of 5000 genes selected for early hematopoietic cells by subtracting a large cDNA library generated from lineage-committed hematopoietic cells from a cDNA library generated from purified HSC/MPP populations. They then compared gene expression of a population enriched for HSC ($\text{Rho}^{\text{low}}\text{KSL}$, $\text{Rhodamine}^{\text{low}}\text{c-Kit}^+\text{Sca-1}^+\text{Lin}^-$) and another enriched for MPP ($\text{Rho}^{\text{hi}}\text{LSK}$). Throughout these screens, 30 HSC ($\text{Rho}^{\text{low}}\text{KSL}$) specific genes, 30 MPP ($\text{Rho}^{\text{hi}}\text{LSK}$) specific genes, and 70 commonly expressed genes were reported [116]. Utilizing Affymetrix type arrays, Ivanova et al compared human fetal liver HSC, LCP (Lineage Committed Progenitor), MBC (Mature Blood Cell); mouse fetal liver HSC, LCP, MBC; and mouse adult LT-HSC, ST-HSC, LCP, MBC. They reported that there were 822 HSC-specific genes conserved between mouse and human. Also, they addressed genetic mechanisms operating in multiple stem cell types by comparing mouse HSC, NSC (neural stem cell), and ES cells, and revealed 283 genes that are commonly expressed among these three stem cell types [117]. The next year, Akashi et al. characterized gene expression patterns according to early lineage commitment by comparing HSC, MPP, CMP, and CLP [118]. Terskikh et al. also characterized expression of 1200 selected mouse genes in 7 early hematopoietic populations (HSC, CMP, MEP, GMP, CLP, pro-T, and pro-B) [78]. These studies revealed successive expression of lineage-specific genes according to hematopoietic hierarchy, and active transcriptional status of HSCs even though they are cell cycle quiescent.

Around 2005, advances in both microarray technology and bioinformatics, such as used in the Gene Ontology project, allowed for further detailed profiling. Forsberg et al. analyzed LT-HSC ($\text{Thy1.1}^{\text{low}}\text{Flk2}^-\text{KSL}$), ST-HSC ($\text{Thy1.1}^{\text{low}}\text{FL}^+\text{KSL}$), and MPP ($\text{Thy1.1}^-\text{Flk2}^+\text{KSL}$) using arrays produced at the Stanford Microarray Facility, covering 42000 genes. From the list of differentially regulated genes, several candidate cell-surface markers were proposed [119]. Later, one of them, the cell adhesion molecule endothelial cell-selective adhesion molecule (Esam1) was shown to be a HSC cell surface marker [120]. To elucidate the mechanisms underlying hematopoietic aging, Rossi et al. compared properties of highly purified HSCs between young mice and old mice by functional assays as well as gene expression profiling. Phenotypic HSC are found in higher frequency in the marrows of 2 years old mice compared to marrows of 2 months old mice [121,122]. They found that in concert with mouse age, HSC increase self-renewal activity and differentiation capacity to generate myeloid lineages, and diminishes lymphoid productivity. Microarray analyses revealed that HSC aging was accompanied by the systemic down-regulation of genes responsible for lymphoid commitment. Moreover, many genes involved in leukemic transformation were up-regulated in HSCs from old mice [122].

Progress in amplification technology has enabled evaluation of gene expression at a single cell level. Ramos et al profiled gene expression of 12 single HSC enriched by sorting with a combination of cell-surface marker expression as $\text{Sca-1}^+\text{Gr-1}^-$ and differential Hoechst dye efflux ability (Side Population, SP), and reported high diversity of gene expression by these individual cells selected from these population [123]. In 2007, Chambers et al. characterized population enriched for HSC, as defined by $\text{SP Sca1}^+\text{c-Kit}^+\text{Lin}^-$ and terminally differentiated populations including erythrocyte, granulocyte, monocytes, NK cells, activated and naïve T- and B-cells, and published lists of genes uniquely expressed in each cell type [124]. Apart from microarray technology, Yashiro et al. performed high-throughput sequencing of cDNA libraries from two different definitions of HSC, $\text{CD34}^-\text{KSL}$ and SP Lin^- cells. After digital subtraction to exclude ubiquitously expressed genes, they reported 25 HSC specific genes including five novel genes as well as 29 miRNA-like non-coding RNAs [125].

As seen in Table 4, microarray platforms used have been rapidly evolving within 6 years. At the same time, with continuous progress in the method for HSC purification, each study uses

different criteria to define 'HSC', and uses a different counterpart cell population for comparison. Thus, it is not yet possible to cross-compare the lists of differentially regulated genes provided by each study. On the other hand, current accumulation of a number of gene profiling experiments on the same platform enables an exhaustive analysis. Recently, Miranda-Saavedra et al. assembled 271 microarray datasets from 15 published studies that compared gene expression profiles of 37 distinct mouse hematopoietic populations from 15 distinct studies already published, and provided an integrated database to browse these gene expressions named "BloodExpress" (<http://hscl.cimr.cam.ac.uk/bloodexpress>). This database will be one of the frameworks to integrate our emerging understanding of the kinetics of gene expressions mapped on the entire representation of the cells in the hematopoietic hierarchy[126]. Just as there is as rapid progress of the microarray technology for gene expression profiling, recently emerging methodologies for systems profiling of epigenetic status, microRNA expression, protein expression, and protein phosphorylation are expected to be applicable to purified HSC in the near future. One such analysis of human hematopoietic stem and progenitor cells has resulted in the beginnings of a systems approach to understand human hematopoietic cell biology [127]

Summary

The blood system reflects the balance of two essential abilities of HSC, self-renewal and differentiation. Intensive studies have revealed the hierarchical structure of the blood system and key molecules regulating HSC. However, the entire picture of the molecular interactions orchestrating HSC fate is yet unclear. Synergies between highly developed biological and molecular approaches and rapidly emerging systems approaches will hopefully integrate and accelerate our understanding of this tiny but mighty HSC population.

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References

1. Ogawa M. Differentiation and proliferation of hematopoietic stem cells. *Blood*. 1993; 81(11):2844–2853. [PubMed: 8499622]
2. Rossi DJ, et al. Hematopoietic stem cell quiescence attenuates DNA damage response and permits DNA damage accumulation during aging. *Cell Cycle*. 2007; 6(19):2371–2376. [PubMed: 17700071]
3. Reya T, et al. Stem cells, cancer, and cancer stem cells. *Nature*. 2001; 414(6859):105–111. [PubMed: 11689955]
4. Weissman I. Stem cell research: paths to cancer therapies and regenerative medicine. *JAMA*. 2005; 294(11):1359–1366. [PubMed: 16174694]
5. Becker AJ, Mc CE, Till JE. Cytological demonstration of the clonal nature of spleen colonies derived from transplanted mouse marrow cells. *Nature*. 1963; 197:452–454. [PubMed: 13970094]
6. Siminovitch L, McCulloch EA, Till JE. The Distribution of Colony-Forming Cells among Spleen Colonies. *J Cell Physiol*. 1963; 62:327–336. [PubMed: 14086156]
7. Till JE, Mc CE. A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. *Radiat Res*. 1961; 14:213–222. [PubMed: 13776896]
8. Wu AM, et al. Cytological evidence for a relationship between normal hematopoietic colony-forming cells and cells of the lymphoid system. *J Exp Med*. 1968; 127(3):455–464. [PubMed: 5636553]
9. Nakorn TN, Miyamoto T, Weissman IL. Characterization of mouse clonogenic megakaryocyte progenitors. *Proc Natl Acad Sci U S A*. 2003; 100(1):205–210. [PubMed: 12490656]

10. Muller-Sieburg CE, Whitlock CA, Weissman IL. Isolation of two early B lymphocyte progenitors from mouse marrow: a committed pre-pre-B cell and a clonogenic Thy-1-lo hematopoietic stem cell. *Cell*. 1986; 44(4):653–662. [PubMed: 2868799]
11. Spangrude GJ, Heimfeld S, Weissman IL. Purification and characterization of mouse hematopoietic stem cells. *Science*. 1988; 241(4861):58–62. [PubMed: 2898810]
12. Uchida N, Weissman IL. Searching for hematopoietic stem cells: evidence that Thy-1.1lo Lin–Sca-1+ cells are the only stem cells in C57BL/Ka-Thy-1.1 bone marrow. *J Exp Med*. 1992; 175(1): 175–184. [PubMed: 1346154]
13. Ikuta K, Weissman IL. Evidence that hematopoietic stem cells express mouse c-kit but do not depend on steel factor for their generation. *Proc Natl Acad Sci U S A*. 1992; 89(4):1502–1506. [PubMed: 1371359]
14. Smith LG, Weissman IL, Heimfeld S. Clonal analysis of hematopoietic stem-cell differentiation in vivo. *Proc Natl Acad Sci U S A*. 1991; 88(7):2788–2792. [PubMed: 1672767]
15. Morrison SJ, Weissman IL. The long-term repopulating subset of hematopoietic stem cells is deterministic and isolatable by phenotype. *Immunity*. 1994; 1(8):661–673. [PubMed: 7541305]
16. Osawa M, et al. Long-term lymphohematopoietic reconstitution by a single CD34-low/negative hematopoietic stem cell. *Science*. 1996; 273(5272):242–245. [PubMed: 8662508]
17. Uchida N, et al. High doses of purified stem cells cause early hematopoietic recovery in syngeneic and allogeneic hosts. *J Clin Invest*. 1998; 101(5):961–966. [PubMed: 9486965]
18. Christensen JL, Weissman IL. Flk-2 is a marker in hematopoietic stem cell differentiation: a simple method to isolate long-term stem cells. *Proc Natl Acad Sci U S A*. 2001; 98(25):14541–14546. [PubMed: 11724967]
19. Kiel MJ, et al. SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. *Cell*. 2005; 121(7):1109–1121. [PubMed: 15989959]
20. Adolfsson J, et al. Identification of Flt3+ lympho-myeloid stem cells lacking erythro-megakaryocytic potential a revised road map for adult blood lineage commitment. *Cell*. 2005; 121(2):295–306. [PubMed: 15851035]
21. Arinobu Y, et al. Reciprocal activation of GATA-1 and PU.1 marks initial specification of hematopoietic stem cells into myeloerythroid and myelolymphoid lineages. *Cell Stem Cell*. 2007; 1(4):416–427. [PubMed: 18371378]
22. Forsberg EC, et al. New evidence supporting megakaryocyte-erythrocyte potential of flk2/flt3+ multipotent hematopoietic progenitors. *Cell*. 2006; 126(2):415–426. [PubMed: 16873070]
23. Serwold T, Ehrlich LI, Weissman IL. Reductive isolation from bone marrow and blood implicates common lymphoid progenitors as the major source of thymopoiesis. *Blood*. 2009; 113(4):807–815. [PubMed: 18927436]
24. Karsunky H, et al. Flk2+ common lymphoid progenitors possess equivalent differentiation potential for the B and T lineages. *Blood*. 2008; 111(12):5562–5570. [PubMed: 18424665]
25. Kondo M, Weissman IL, Akashi K. Identification of clonogenic common lymphoid progenitors in mouse bone marrow. *Cell*. 1997; 91(5):661–672. [PubMed: 9393859]
26. Akashi K, et al. A clonogenic common myeloid progenitor that gives rise to all myeloid lineages. *Nature*. 2000; 404(6774):193–197. [PubMed: 10724173]
27. Pronk CJ, et al. Elucidation of the phenotypic, functional, and molecular topography of a myeloerythroid progenitor cell hierarchy. *Cell Stem Cell*. 2007; 1(4):428–442. [PubMed: 18371379]
28. Traver D, et al. Development of CD8alpha-positive dendritic cells from a common myeloid progenitor. *Science*. 2000; 290(5499):2152–2154. [PubMed: 11118150]
29. Manz MG, et al. Dendritic cell development from common myeloid progenitors. *Ann N Y Acad Sci*. 2001; 938:167–173. discussion 173-4. [PubMed: 11458504]
30. Manz MG, et al. Dendritic cell potentials of early lymphoid and myeloid progenitors. *Blood*. 2001; 97(11):3333–3341. [PubMed: 11369621]
31. Baum CM, et al. Isolation of a candidate human hematopoietic stem-cell population. *Proc Natl Acad Sci U S A*. 1992; 89(7):2804–2808. [PubMed: 1372992]

32. McCune JM, et al. The SCID-hu mouse: murine model for the analysis of human hematolymphoid differentiation and function. *Science*. 1988; 241(4873):1632–1639. [PubMed: 2971269]
33. Civin CI, et al. Antigenic analysis of hematopoiesis. III. A hematopoietic progenitor cell surface antigen defined by a monoclonal antibody raised against KG-1a cells. *J Immunol*. 1984; 133(1): 157–165. [PubMed: 6586833]
34. DiGiusto D, et al. Human fetal bone marrow early progenitors for T, B, and myeloid cells are found exclusively in the population expressing high levels of CD34. *Blood*. 1994; 84(2):421–432. [PubMed: 7517715]
35. Krause DS, et al. CD34: structure, biology, and clinical utility. *Blood*. 1996; 87(1):1–13. [PubMed: 8547630]
36. Hao QL, et al. Extended long-term culture reveals a highly quiescent and primitive human hematopoietic progenitor population. *Blood*. 1996; 88(9):3306–3313. [PubMed: 8896394]
37. Huang S, Terstappen LW. Lymphoid and myeloid differentiation of single human CD34+, HLA-DR+, CD38– hematopoietic stem cells. *Blood*. 1994; 83(6):1515–1526. [PubMed: 7510144]
38. Miller JS. Single adult human CD34(+)/Lin–/CD38(–) progenitors give rise to natural killer cells, B-lineage cells, dendritic cells, and myeloid cells. *Blood*. 1999; 93(1):96–106. [PubMed: 9864151]
39. Uchida N, et al. HIV, but not murine leukemia virus, vectors mediate high efficiency gene transfer into freshly isolated G0/G1 human hematopoietic stem cells. *Proc Natl Acad Sci U S A*. 1998; 95(20):11939–11944. [PubMed: 9751769]
40. Petzer AL, et al. Self-renewal of primitive human hematopoietic cells (long-term-culture-initiating cells) in vitro and their expansion in defined medium. *Proc Natl Acad Sci U S A*. 1996; 93(4): 1470–1474. [PubMed: 8643656]
41. Bhatia M, et al. Purification of primitive human hematopoietic cells capable of repopulating immune-deficient mice. *Proc Natl Acad Sci U S A*. 1997; 94(10):5320–5325. [PubMed: 9144235]
42. Larochelle A, et al. Identification of primitive human hematopoietic cells capable of repopulating NOD/SCID mouse bone marrow: implications for gene therapy. *Nat Med*. 1996; 2(12):1329–1337. [PubMed: 8946831]
43. Michallet M, et al. Transplantation with selected autologous peripheral blood CD34+Thy1+ hematopoietic stem cells (HSCs) in multiple myeloma: impact of HSC dose on engraftment, safety, and immune reconstitution. *Exp Hematol*. 2000; 28(7):858–870. [PubMed: 10907648]
44. Negrin RS, et al. Transplantation of highly purified CD34+Thy-1+ hematopoietic stem cells in patients with metastatic breast cancer. *Biol Blood Marrow Transplant*. 2000; 6(3):262–271. [PubMed: 10871151]
45. Vose JM, et al. Transplantation of highly purified CD34+Thy-1+ hematopoietic stem cells in patients with recurrent indolent non-Hodgkin's lymphoma. *Biol Blood Marrow Transplant*. 2001; 7(12):680–687. [PubMed: 11787531]
46. Guenechea G, et al. Distinct classes of human stem cells that differ in proliferative and self-renewal potential. *Nat Immunol*. 2001; 2(1):75–82. [PubMed: 11135582]
47. Mazurier F, et al. Lentivector-mediated clonal tracking reveals intrinsic heterogeneity in the human hematopoietic stem cell compartment and culture-induced stem cell impairment. *Blood*. 2004; 103(2):545–552. [PubMed: 14504079]
48. McKenzie JL, et al. Individual stem cells with highly variable proliferation and self-renewal properties comprise the human hematopoietic stem cell compartment. *Nat Immunol*. 2006; 7(11): 1225–1233. [PubMed: 17013390]
49. Majeti R, Park CY, Weissman IL. Identification of a hierarchy of multipotent hematopoietic progenitors in human cord blood. *Cell Stem Cell*. 2007; 1(6):635–645. [PubMed: 18371405]
50. Fritsch G, et al. Rapid discrimination of early CD34+ myeloid progenitors using CD45-RA analysis. *Blood*. 1993; 81(9):2301–2309. [PubMed: 7683216]
51. Galy A, et al. Human T, B, natural killer, and dendritic cells arise from a common bone marrow progenitor cell subset. *Immunity*. 1995; 3(4):459–473. [PubMed: 7584137]
52. Kimura T, et al. Simultaneous activation of signals through gp130, c-kit, and interleukin-3 receptor promotes a trilineage blood cell production in the absence of terminally acting lineage-specific factors. *Blood*. 1997; 90(12):4767–4778. [PubMed: 9389693]

53. Manz MG, et al. Prospective isolation of human clonogenic common myeloid progenitors. *Proc Natl Acad Sci U S A*. 2002; 99(18):11872–11877. [PubMed: 12193648]
54. Russell ES. Analysis of pleiotropism at the W-locus in the mouse; relationship between the effects of W and Wv substitution on hair pigmentation and on erythrocytes. *Genetics*. 1949; 34(6):708–723. [PubMed: 15393917]
55. Sarvella PA, Russell LB. Steel, a new dominant gene in the mouse. *J. Hered.* 1956; 47:123–128.
56. Bernstein SE, Russell ES, Keighley G. Two hereditary mouse anemias deficient in response to erythropoietin. *Ann. NY Acad. Sci.* 1968; 149:475–485. [PubMed: 5240740]
57. Ebbe S, Phalen E, Stohlman F Jr. Abnormalities of megakaryocytes in S1-S1d mice. *Blood*. 1973; 42(6):865–871. [PubMed: 4759914]
58. Ebbe S, Phalen E, Stohlman F Jr. Abnormalities of megakaryocytes in W-WV mice. *Blood*. 1973; 42(6):857–864. [PubMed: 4759913]
59. Ruscetti FW, et al. Reduced blood and marrow neutrophils and granulocytic colony-forming cells in S1/S1d mice. *Proc Soc Exp Biol Med*. 1976; 152(3):398–402. [PubMed: 1084999]
60. Kitamura Y, Go S. Decreased production of mast cells in S1/S1d anemic mice. *Blood*. 1979; 53(3):492–497. [PubMed: 367470]
61. Kitamura Y, Go S, Hatanaka K. Decrease of mast cells in W/Wv mice and their increase by bone marrow transplantation. *Blood*. 1978; 52(2):447–452. [PubMed: 352443]
62. Geissler EN, Russell ES. Analysis of the hematopoietic effects of new dominant spotting (W) mutations of the mouse. II. Effects on mast cell development. *Exp Hematol*. 1983; 11(6):461–466. [PubMed: 6352298]
63. Fujita J, et al. In vitro duplication and in vivo cure of mast-cell deficiency of S1/S1d mutant mice by cloned 3T3 fibroblasts. *Proc Natl Acad Sci U S A*. 1989; 86(8):2888–2891. [PubMed: 2704751]
64. Mayer TC, Green MC. An experimental analysis of the pigment defect caused by mutations at the W and S1 loci in mice. *Dev Biol*. 1968; 18(1):62–75. [PubMed: 4876023]
65. Russell ES, Bernstein SE. Proof of whole-cell implant in therapy of W-series anemia. *Arch Biochem Biophys*. 1968; 125(2):594–597. [PubMed: 4872322]
66. Jarboe DL, Huff TF. The mast cell-committed progenitor. II. W/Wv mice do not make mast cell-committed progenitors and S1/S1d fibroblasts do not support development of normal mast cell-committed progenitors. *J Immunol*. 1989; 142(7):2418–2423. [PubMed: 2926139]
67. Nocka K, et al. Expression of c-kit gene products in known cellular targets of W mutations in normal and W mutant mice—evidence for an impaired c-kit kinase in mutant mice. *Genes Dev*. 1989; 3(6):816–826. [PubMed: 2473008]
68. Reith AD, et al. W mutant mice with mild or severe developmental defects contain distinct point mutations in the kinase domain of the c-kit receptor. *Genes Dev*. 1990; 4(3):390–400. [PubMed: 1692559]
69. Zsebo KM, et al. Stem cell factor is encoded at the Sl locus of the mouse and is the ligand for the c-kit tyrosine kinase receptor. *Cell*. 1990; 63(1):213–224. [PubMed: 1698556]
70. Whitlock CA, et al. Bone marrow stromal cell lines with lymphopoietic activity express high levels of a pre-B neoplasia-associated molecule. *Cell*. 1987; 48(6):1009–1021. [PubMed: 3493849]
71. Zhang J, et al. Identification of the haematopoietic stem cell niche and control of the niche size. *Nature*. 2003; 425(6960):836–841. [PubMed: 14574412]
72. Calvi LM, et al. Osteoblastic cells regulate the haematopoietic stem cell niche. *Nature*. 2003; 425(6960):841–846. [PubMed: 14574413]
73. Adolfsson J, et al. Upregulation of Flt3 expression within the bone marrow Lin(–)Sca1(+)c-kit(+) stem cell compartment is accompanied by loss of self-renewal capacity. *Immunity*. 2001; 15(4):659–669. [PubMed: 11672547]
74. Buza-Vidas N, et al. Cytokines regulate postnatal hematopoietic stem cell expansion: opposing roles of thrombopoietin and LNK. *Genes Dev*. 2006; 20(15):2018–2023. [PubMed: 16882979]
75. Kimura S, et al. Hematopoietic stem cell deficiencies in mice lacking c-Mpl, the receptor for thrombopoietin. *Proc Natl Acad Sci U S A*. 1998; 95(3):1195–1200. [PubMed: 9448308]
76. Solar GP, et al. Role of c-mpl in early hematopoiesis. *Blood*. 1998; 92(1):4–10. [PubMed: 9639492]

77. Yang L, et al. Identification of Lin(-)Sca1(+)kit(+)/CD34(+)/Flt3- short-term hematopoietic stem cells capable of rapidly reconstituting and rescuing myeloablated transplant recipients. *Blood*. 2005; 105(7):2717–2723. [PubMed: 15572596]
78. Terskikh AV. Gene expression analysis of purified hematopoietic stem cells and committed progenitors. *Blood*. 2003; 102(1):94–101. [PubMed: 12623852]
79. Seita J, et al. Lnk negatively regulates self-renewal of hematopoietic stem cells by modifying thrombopoietin-mediated signal transduction. *Proc Natl Acad Sci U S A*. 2007; 104(7):2349–2354. [PubMed: 17284614]
80. Takaki S, et al. Control of B cell production by the adaptor protein lnk. Definition Of a conserved family of signal-modulating proteins. *Immunity*. 2000; 13(5):599–609. [PubMed: 11114373]
81. Tong W, Zhang J, Lodish HF. Lnk inhibits erythropoiesis and Epo-dependent JAK2 activation and downstream signaling pathways. *Blood*. 2005; 105(12):4604–4612. [PubMed: 15705783]
82. Velazquez L, et al. Cytokine signaling and hematopoietic homeostasis are disrupted in Lnk-deficient mice. *J Exp Med*. 2002; 195(12):1599–1611. [PubMed: 12070287]
83. Ema H, et al. Quantification of self-renewal capacity in single hematopoietic stem cells from normal and Lnk-deficient mice. *Dev Cell*. 2005; 8(6):907–914. [PubMed: 15935779]
84. Nandurkar HH, et al. Adult mice with targeted mutation of the interleukin-11 receptor (IL11Ra) display normal hematopoiesis. *Blood*. 1997; 90(6):2148–2159. [PubMed: 9310465]
85. Willert K, et al. Wnt proteins are lipid-modified and can act as stem cell growth factors. *Nature*. 2003; 423(6938):448–452. [PubMed: 12717451]
86. Reya T, et al. A role for Wnt signalling in self-renewal of haematopoietic stem cells. *Nature*. 2003; 423(6938):409–414. [PubMed: 12717450]
87. Kirstetter P, et al. Activation of the canonical Wnt pathway leads to loss of hematopoietic stem cell repopulation and multilineage differentiation block. *Nat Immunol*. 2006; 7(10):1048–1056. [PubMed: 16951689]
88. Scheller M, et al. Hematopoietic stem cell and multilineage defects generated by constitutive beta-catenin activation. *Nat Immunol*. 2006; 7(10):1037–1047. [PubMed: 16951686]
89. Cobas M, et al. Beta-catenin is dispensable for hematopoiesis and lymphopoiesis. *J Exp Med*. 2004; 199(2):221–229. [PubMed: 14718516]
90. Jeannot G, et al. Long-term, multilineage hematopoiesis occurs in the combined absence of beta-catenin and gamma-catenin. *Blood*. 2008; 111(1):142–149. [PubMed: 17906078]
91. Koch U, et al. Simultaneous loss of beta- and gamma-catenin does not perturb hematopoiesis or lymphopoiesis. *Blood*. 2008; 111(1):160–164. [PubMed: 17855627]
92. Zhao C, et al. Loss of beta-catenin impairs the renewal of normal and CML stem cells in vivo. *Cancer Cell*. 2007; 12(6):528–541. [PubMed: 18068630]
93. Batar P, et al. TGF-(beta)1 maintains hematopoietic immaturity by a reversible negative control of cell cycle and induces CD34 antigen up-modulation. *J Cell Sci*. 2000; 113(Pt 3):383–390. [PubMed: 10639326]
94. Garbe A, et al. Transforming growth factor-beta 1 delays formation of granulocyte-macrophage colony-forming cells, but spares more primitive progenitors during ex vivo expansion of CD34+ haemopoietic progenitor cells. *Br J Haematol*. 1997; 99(4):951–958. [PubMed: 9432049]
95. Sitnicka E, et al. Transforming growth factor beta 1 directly and reversibly inhibits the initial cell divisions of long-term repopulating hematopoietic stem cells. *Blood*. 1996; 88(1):82–88. [PubMed: 8704205]
96. Fortunel N, et al. High proliferative potential-quiescent cells: a working model to study primitive quiescent hematopoietic cells. *J Cell Sci*. 1998; 111(Pt 13):1867–1875. [PubMed: 9625749]
97. Hatzfeld J, et al. Release of early human hematopoietic progenitors from quiescence by antisense transforming growth factor beta 1 or Rb oligonucleotides. *J Exp Med*. 1991; 174(4):925–929. [PubMed: 1717634]
98. Soma T, Yu JM, Dunbar CE. Maintenance of murine long-term repopulating stem cells in ex vivo culture is affected by modulation of transforming growth factor-beta but not macrophage inflammatory protein-1 alpha activities. *Blood*. 1996; 87(11):4561–4567. [PubMed: 8639824]

99. Cheng T, et al. Transforming growth factor beta 1 mediates cell-cycle arrest of primitive hematopoietic cells independent of p21(Cip1/Waf1) or p27(Kip1). *Blood*. 2001; 98(13):3643–3649. [PubMed: 11739168]
100. Dao MA, Hwa J, Nolte JA. Molecular mechanism of transforming growth factor beta-mediated cell-cycle modulation in primary human CD34(+) progenitors. *Blood*. 2002; 99(2):499–506. [PubMed: 11781230]
101. Dao MA, Taylor N, Nolte JA. Reduction in levels of the cyclin-dependent kinase inhibitor p27(kip-1) coupled with transforming growth factor beta neutralization induces cell-cycle entry and increases retroviral transduction of primitive human hematopoietic cells. *Proc Natl Acad Sci U S A*. 1998; 95(22):13006–13011. [PubMed: 9789031]
102. Dubois CM, et al. Transforming growth factor beta is a potent inhibitor of interleukin 1 (IL-1) receptor expression: proposed mechanism of inhibition of IL-1 action. *J Exp Med*. 1990; 172(3):737–744. [PubMed: 2143773]
103. Dubois CM, et al. Transforming growth factor-beta regulates c-kit message stability and cell-surface protein expression in hematopoietic progenitors. *Blood*. 1994; 83(11):3138–3145. [PubMed: 7514900]
104. Ducos K, et al. p21(cip1) mRNA is controlled by endogenous transforming growth factor-beta1 in quiescent human hematopoietic stem/progenitor cells. *J Cell Physiol*. 2000; 184(1):80–85. [PubMed: 10825236]
105. Jacobsen SE, et al. Bidirectional effects of transforming growth factor beta (TGF-beta) on colony-stimulating factor-induced human myelopoiesis in vitro: differential effects of distinct TGF-beta isoforms. *Blood*. 1991; 78(9):2239–2247. [PubMed: 1718492]
106. Jacobsen SE, et al. Transforming growth factor-beta trans-modulates the expression of colony stimulating factor receptors on murine hematopoietic progenitor cell lines. *Blood*. 1991; 77(8):1706–1716. [PubMed: 1707695]
107. Sansilvestri P, et al. Early CD34^{high} cells can be separated into KIT^{high} cells in which transforming growth factor-beta (TGF-beta) downmodulates c-kit and KIT^{low} cells in which anti-TGF-beta upmodulates c-kit. *Blood*. 1995; 86(5):1729–1735. [PubMed: 7544639]
108. Scandura JM, et al. Transforming growth factor beta-induced cell cycle arrest of human hematopoietic cells requires p57KIP2 up-regulation. *Proc Natl Acad Sci U S A*. 2004; 101(42):15231–15236. [PubMed: 15477587]
109. Yamazaki S, et al. TGF-beta as a candidate bone marrow niche signal to induce hematopoietic stem cell hibernation. *Blood*. 2009; 113(6):1250–1256. [PubMed: 18945958]
110. Larsson J, et al. TGF-beta signaling-deficient hematopoietic stem cells have normal self-renewal and regenerative ability in vivo despite increased proliferative capacity in vitro. *Blood*. 2003; 102(9):3129–3135. [PubMed: 12842983]
111. Dumont DJ, et al. tek, a novel tyrosine kinase gene located on mouse chromosome 4, is expressed in endothelial cells and their presumptive precursors. *Oncogene*. 1992; 7(8):1471–1480. [PubMed: 1630810]
112. Iwama A, et al. Molecular cloning and characterization of mouse TIE and TEK receptor tyrosine kinase genes and their expression in hematopoietic stem cells. *Biochem Biophys Res Commun*. 1993; 195(1):301–309. [PubMed: 8395828]
113. Sato TN, et al. Tie-1 and tie-2 define another class of putative receptor tyrosine kinase genes expressed in early embryonic vascular system. *Proc Natl Acad Sci U S A*. 1993; 90(20):9355–9358. [PubMed: 8415706]
114. Puri MC, Bernstein A. Requirement for the TIE family of receptor tyrosine kinases in adult but not fetal hematopoiesis. *Proc Natl Acad Sci U S A*. 2003; 100(22):12753–12758. [PubMed: 14530387]
115. Arai F, et al. Tie2/angiopoietin-1 signaling regulates hematopoietic stem cell quiescence in the bone marrow niche. *Cell*. 2004; 118(2):149–161. [PubMed: 15260986]
116. Park IK, et al. Differential gene expression profiling of adult murine hematopoietic stem cells. *Blood*. 2002; 99(2):488–498. [PubMed: 11781229]
117. Ivanova NB, et al. A stem cell molecular signature. *Science*. 2002; 298(5593):601–604. [PubMed: 12228721]

118. Akashi K, et al. Transcriptional accessibility for genes of multiple tissues and hematopoietic lineages is hierarchically controlled during early hematopoiesis. *Blood*. 2003; 101(2):383–389. [PubMed: 12393558]
119. Forsberg EC, et al. Differential expression of novel potential regulators in hematopoietic stem cells. *PLoS Genet*. 2005; 1(3):e28. [PubMed: 16151515]
120. Ooi AG, et al. The adhesion molecule *esam1* is a novel hematopoietic stem cell marker. *Stem Cells*. 2009; 27(3):653–661. [PubMed: 19074415]
121. Morrison SJ, et al. The aging of hematopoietic stem cells. *Nat Med*. 1996; 2(9):1011–1016. [PubMed: 8782459]
122. Rossi DJ, et al. Cell intrinsic alterations underlie hematopoietic stem cell aging. *Proc Natl Acad Sci U S A*. 2005; 102(26):9194–9199. [PubMed: 15967997]
123. Ramos CA, et al. Evidence for diversity in transcriptional profiles of single hematopoietic stem cells. *PLoS Genet*. 2006; 2(9):e159. [PubMed: 17009876]
124. Chambers SM, et al. Hematopoietic fingerprints: an expression database of stem cells and their progeny. *Cell Stem Cell*. 2007; 1(5):578–591. [PubMed: 18371395]
125. Yashiro Y, et al. Transcriptional profiling of hematopoietic stem cells by high-throughput sequencing. *Int J Hematol*. 2009; 89(1):24–33. [PubMed: 19050837]
126. Miranda-Saavedra D, et al. BloodExpress: a database of gene expression in mouse haematopoiesis. *Nucleic Acids Res*. 2009; 37(Database issue):D873–D879. [PubMed: 18987008]
127. Majeti R, et al. Dysregulated gene expression networks in human acute myelogenous leukemia stem cells. *Proc Natl Acad Sci U S A*. 2009; 106(9):3396–3401. [PubMed: 19218430]
128. de Sauvage FJ, et al. Physiological regulation of early and late stages of megakaryocytopoiesis by thrombopoietin. *J Exp Med*. 1996; 183(2):651–656. [PubMed: 8627177]
129. Suri C, et al. Requisite role of angiopoietin-1, a ligand for the TIE2 receptor, during embryonic angiogenesis. *Cell*. 1996; 87(7):1171–1180. [PubMed: 8980224]
130. Mach N, et al. Involvement of interleukin-3 in delayed-type hypersensitivity. *Blood*. 1998; 91(3):778–783. [PubMed: 9446636]
131. Kopf M, et al. Impaired immune and acute-phase responses in interleukin-6-deficient mice. *Nature*. 1994; 368(6469):339–342. [PubMed: 8127368]
132. Shull MM, et al. Targeted disruption of the mouse transforming growth factor-beta 1 gene results in multifocal inflammatory disease. *Nature*. 1992; 359(6397):693–699. [PubMed: 1436033]
133. Dickson MC, et al. Defective haematopoiesis and vasculogenesis in transforming growth factor-beta 1 knock out mice. *Development*. 1995; 121(6):1845–1854. [PubMed: 7600998]
134. Sanford LP, et al. TGFbeta2 knockout mice have multiple developmental defects that are non-overlapping with other TGFbeta knockout phenotypes. *Development*. 1997; 124(13):2659–2670. [PubMed: 9217007]
135. Kaartinen V, et al. Abnormal lung development and cleft palate in mice lacking TGF-beta 3 indicates defects of epithelial-mesenchymal interaction. *Nat Genet*. 1995; 11(4):415–421. [PubMed: 7493022]
136. Gurney AL, et al. Thrombocytopenia in *c-mpl*-deficient mice. *Science*. 1994; 265(5177):1445–1447. [PubMed: 8073287]
137. Dumont DJ, et al. Dominant-negative and targeted null mutations in the endothelial receptor tyrosine kinase, *tek*, reveal a critical role in vasculogenesis of the embryo. *Genes Dev*. 1994; 8(16):1897–1909. [PubMed: 7958865]
138. Sato TN, et al. Distinct roles of the receptor tyrosine kinases Tie-1 and Tie-2 in blood vessel formation. *Nature*. 1995; 376(6535):70–74. [PubMed: 7596437]
139. Ichihara M, et al. Impaired interleukin-3 (IL-3) response of the A/J mouse is caused by a branch point deletion in the IL-3 receptor alpha subunit gene. *EMBO J*. 1995; 14(5):939–950. [PubMed: 7889941]
140. Nishinakamura R, et al. Hematopoiesis in mice lacking the entire granulocyte-macrophage colony-stimulating factor/interleukin-3/interleukin-5 functions. *Blood*. 1996; 88(7):2458–2464. [PubMed: 8839836]

141. Yoshida K, et al. Targeted disruption of gp130, a common signal transducer for the interleukin 6 family of cytokines, leads to myocardial and hematological disorders. *Proc Natl Acad Sci U S A*. 1996; 93(1):407–411. [PubMed: 8552649]
142. Larsson J, et al. Abnormal angiogenesis but intact hematopoietic potential in TGF-beta type I receptor-deficient mice. *EMBO J*. 2001; 20(7):1663–1673. [PubMed: 11285230]
143. Oshima M, Oshima H, Taketo MM. TGF-beta receptor type II deficiency results in defects of yolk sac hematopoiesis and vasculogenesis. *Dev Biol*. 1996; 179(1):297–302. [PubMed: 8873772]

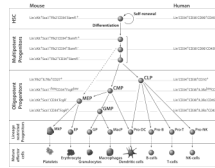


Figure 1. Model of the hematopoietic hierarchy

The HSC resides at the top of the hierarchy, and is defined as the cell that has both the self-renewal capacity and the potential to give rise to all hematopoietic cell types (multipotency). Throughout differentiation, a HSC first loses self-renewal capacity, then loses lineage potential step-by-step as it commits to become a mature functional cell of a certain lineage. The cell surface phenotype of each population is shown for the mouse and human systems. Intermediate precursors between the first lineage committed progenitors and final mature cell, and different subsets of mature B- and T-cells are omitted. In the mouse system, heterogeneity of MPPs has been revealed by differences in cell surface marker phenotypes and functional differences of their subsets discussed. For example, evidence suggests that some of MPPs directly give rise to MEP without passing through CMP (dashed arrow). HSC: hematopoietic stem cell, CLP: common lymphoid progenitor, CMP: common myeloid progenitor, MEP: Megakaryocyte/erythrocyte progenitor, GMP: granulocyte/macrophage progenitor, Mkp: Megakaryocyte progenitor, EP: erythrocyte progenitor, GP: granulocyte progenitor, MacP: macrophage progenitor, DC: dendritic cell, NK: natural killer, Lin: lineage markers.

Table 1

Designations used to Define Differentiation Potential of a Cell Populations.

Designation	Differentiation potential implied by designation	Examples of Stem/Progenitors with these Properties
Toti-potent	All embryonic and extraembryonic tissues	zygote
Pluri-potent	All embryonic tissues	ICM, ES cell, iPS cell
Multi-potent	All lineages of a tissue/organ	HSC, NSC
Oligo-potent	Several but not all lineages of a tissue/organ	CMP, CLP
Uni-potent	Single lineage of a tissue/organ	Macrophage progenitor

Abbreviations used in Table 1: ICM: inner cell mass, ES: embryonic stem, iPS: induced pluripotent stem, HSC: hematopoietic stem cell, NSC: neural stem cell, CMP: common myeloid progenitor, CLP: common lymphoid progenitor

Table 2

Phenotypes of Cytokine KO mice

Cytokine	Receptor	Fetal Development	Phenotype on Blood System in Adult	Number of HSC in adult	Ref
SCF*	c-kit	Normal	Macrocytic anemia	Decrease	See text
TPO	c-Mpl	Normal	Thrombocytopenia	Decrease	[128]
Ang-1	Tie-2	Lethal (E 12.5)	N/A	See text	[129]
IL-3	IL3Ra + Csfl2rb	Normal	Lack of response to infection in Mast Cells	Normal	[130]
IL-6	IL6R + Il6st	Normal	Decrease of T-cells in peripheral blood	Normal	[131]
IL-11	Il11Ra + Il6st	No report of KO mice			-
TGF-b1	TGFbr1 + TGFbr2	Lethal (E 10.5) ~ Normal	Significant increase of monocyte & neutrophil		[132,133]
TGF-b2	TGFbr1 + TGFbr2	Born with multiple defects	Not evaluated	Not evaluated	[134]
TGF-b3	TGFbr1 + TGFbr2	Born with abnormal lung development and cleft palate	Not evaluated	Not evaluated	[135]

* SI mutant mice

N/A: not applicable

Table 3

Phenotypes of Receptor KO mice

Receptor	Fetal Development	Phenotype on Blood System in Adult	Number of HSC in Adult	Ref
c-Kit *	Lethal (postimplantation) ~ Normal	Macrocytic anemia	Decrease	See text
c-Mpl	Normal	Thrombocytopenia	Decrease	[136]
Tie-2	Lethal (E 10.5)	N/A	N/A	[137,138]
Il3Ra	Normal	Normal	Normal	[139]
Csf2rb	Normal	Acidocytopenia	Normal	[140]
Il6st (gp130)	Lethal (E 12.5~term)	N/A	N/A	[141]
Il11Ra	Normal	Normal	Normal	[84]
Tgfbr1	Lethal (E 10.5)	N/A	See text	[142]
Tgfbr2	Lethal (E 10.5)	N/A	N/A	[143]

* W mutant mice

N/A: not applicable

Table 4

Studies of Mouse Adult HSC Gene Expression Profiling

Year	Microarray platform	HSC	Compare to	Ref
2002	Homemade	Rho ^{low} KLS	Rho ^{hi} KLS	[116]
2002	Affymetrix MU-U74-2 A~C	Rho ^{low} KLS	Various type of cells	[117]
2003	Affymetrix MU-U74-2 A~B	Thy1.1 ^{low} Rho ^{low} KLS	MPP CMP CLP	[118]
2003	Clontech Atlas Mouse cDNA array	Thy1.1 ^{low} KLS	CMP CLP GMP MEP ProT ProB	[78]
2005	Stanford Micoarray Facility 42k mouse cDNA array	Thy1.1 ^{low} Flk2 ⁻ KLS	Thy1.1 ^{low} Flk2 ⁺ KLS Thy1.1 ⁻ Flk2 ⁺ KLS	[119]
2005	Affymetrix Mouse 430 2.0	CD34 ⁻ FL ⁻ KLS (Young)	CD34 ⁻ FL ⁻ KLS (Old)	[122]
2006	Affymetrix Mouse U74A	SP Sca1 ⁺ Gr1 ⁻	CD8 T-cell	[123]
2007	Affymetrix Mouse 430 2.0	SP Sca1 ⁺ c-Kit ⁺ Lin ⁻	Erythrocyte Granulocyte Native T Activated T B cell Monocyte NK	[124]
2008	High throughput sequencing and Digital subtraction	CD34 ⁻ KSL and SP Lin ⁻		[125]

Abbreviations used in this table; Rho: Rhodamine, KLS: c-kit⁺ Lin⁻ Sca-1⁺, SP: Side Population