Cell Stem Cell
Review

Hematopoiesis: A Human Perspective

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DOI 10.1016/j.stem.2012.01.006

Despite its complexity, blood is probably the best understood developmental system, largely due to seminal experimentation in the mouse. Clinically, hematopoietic stem cell (HSC) transplantation represents the most widely deployed regenerative therapy, but human HSCs have only been characterized relatively recently. The discovery that immune-deficient mice could be engrafted with human cells provided a powerful approach for studying HSCs. We highlight 2 decades of studies focusing on isolation and molecular regulation of human HSCs, therapeutic applications, and early lineage commitment steps, and compare mouse and humanized models to identify both conserved and species-specific mechanisms that will aid future preclinical research.

Introduction

Blood is one of the most highly regenerative tissues, with approximately one trillion (10^{12}) cells arising daily in adult human bone marrow (BM). Early anatomists examining the BM noted a wide variety of cellular morphologies corresponding to cells of various blood lineages and stages of differentiation. To explain this diversity, Russian biologist A. Maximow astutely postulated that hematopoiesis is organized as a cellular hierarchy derived from a common precursor, a hematopoietic stem cell (HSC) (Maximow, 1909). The best evidence for the existence of HSCs came during the atomic era. The lethal consequence of radiation was found to be due to BM failure, but exposed recipients could be rescued following injection of spleen or marrow cells from unirradiated donors (Lorenz et al., 1951). Although these studies firmly established the existence of blood-forming cells and the benefits of regenerating the blood system upon HSC transplantation (HSCT), they could not resolve whether there were multiple stem cells restricted to each blood lineage, or whether a single multipotential HSC existed.

The study of hematopoiesis moved from observational to functional when Till and McCulloch showed that the regenerative potential of HSCs could be assayed with clonal in vivo repopulation assays, thus establishing the existence of multipotential HSCs (Becker et al., 1963; Till and McCulloch, 1961). This finding stimulated others to develop clonal in vitro assays that, combined with the advent of a wide array of cell surface antibodies and flow sorting, have culminated in today's finely detailed view of the blood system as a developmental hierarchy with multipotent HSCs at the apex and terminally differentiated cells on the bottom. HSCs are critical for lifelong blood production and are uniquely defined by their capacity to durably self-renew, or generate daughter stem cells, while still contributing to the pool of differentiating cells. As they differentiate, HSCs give rise to a series of progenitor cell intermediates that undergo a gradual fate restriction to assume the identity of a mature blood cell. Lineage relationships between stem cells, progenitors, and mature cells form a complex "roadmap" that can guide investigations of the molecular basis for these developmental transitions. Much of our understanding of hematopoiesis comes from the mouse because, operationally, HSCs can only be identified and measured with functional repopulation assays, raising an obvious barrier to studying human HSCs. However, with the advent of xenotransplantation, robust in vitro clonal assays, and refined sorting strategies, significant progress toward defining the human blood hierarchy has been made. We will divide this review into three parts, the first describing the advances in purification of human HSCs, the second focusing on the molecular regulation of human HSCs and how it can be harnessed for therapy, and the third on how human lineage commitment occurs.

Purification and Clonal Analysis of Human HSCs

The Importance of Combined Mouse and Human Studies

Since the seminal experiments demonstrating that blood lineages are derived from multipotent cells that form macroscopic colonies in the spleen (CFU-S) following transplantation (Till and McCulloch, 1961), the mouse has become an indispensable model system for studying normal and malignant hematopoiesis. Genetic approaches that direct loss or gain of gene function to precisely defined cellular compartments have identified the basic developmental principles that control the emergence of hemogenic tissues during ontogeny and maintain lifelong hematopoiesis in the adult. The molecular regulation of HSCs elucidated from studies in the mouse is documented in a number of reviews (Orkin and Zon, 2008). Despite these advances, the need to complement mouse studies with those in primary human cells has been driven by the growing appreciation for species-specific differences in basic biology and hematology, and their more direct relevance in developing therapeutics. Mouse strains used in research are inbred, and it is often difficult to predict how the choice of a specific genetic background can influence the observed phenotype. By contrast, human populations are genetically diverse, and this variation becomes an intrinsic parameter in human studies that experimental models must take into account. Mice and humans differ in size, ecology, lifespan, and age to reproductive maturity, imposing different selective trade-offs in dealing with tumorigenesis, genotoxic stress, telomerase function, and other factors. Larger body size increases the proliferative demand on human stem and progenitor cells, altering the balance between self-renewal and differentiation, as well

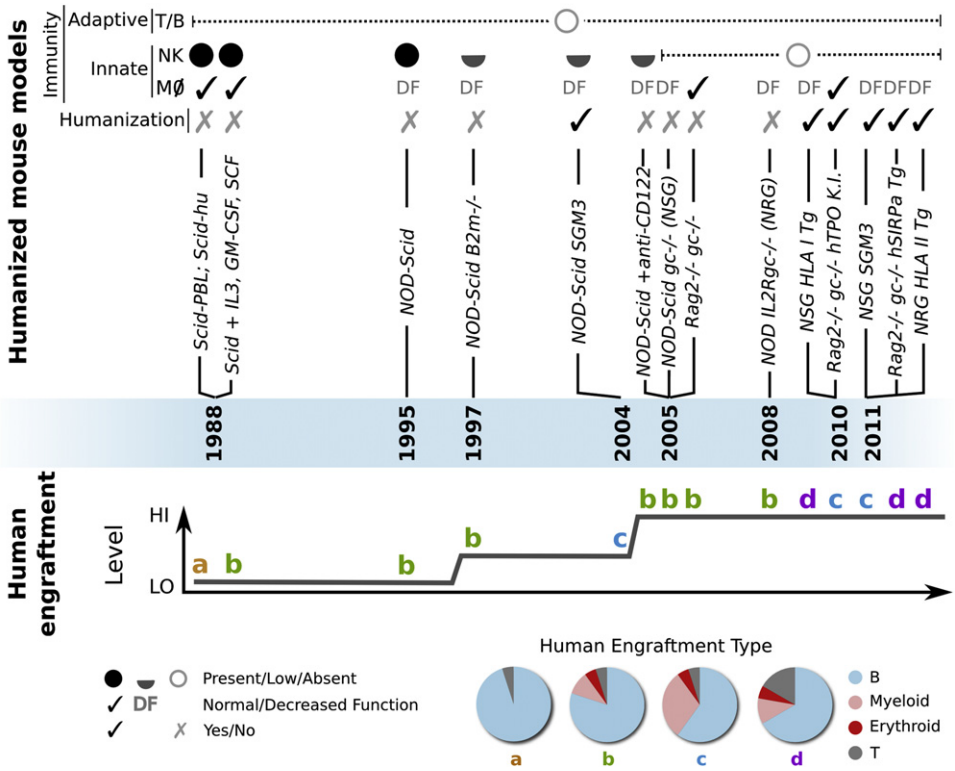

Figure 1. Timeline for the Development of Immune-Deficient Mouse Models

The genotypes of immune-deficient mouse strains are ordered chronologically. The upper panel shows the extent of immunodeficiency and humanization of each model. Humanization is achieved by expressing human proteins as purified protein, as purified transgenes, or from the locus of their mouse homolog (knockin, K.I.). To overcome the limitations due to poor cross-reactivity between mouse and human cytokines, mice that transgenically (Tg) produce human SCF, GM-CSF, and IL-3 (SGM3 mice), or that have the human *TPO* replacing the mouse locus, have been produced. Other humanization strategies include reducing human graft rejection by constitutively expressing human SIRPα, or increasing human T cell function by constitutive expression of human HLA class I or class II. These models are described in greater detail elsewhere (Shultz et al., 2007; Willinger et al., 2011). The lower panel depicts the relative extent of support for human cells achieved in each strain. The general level of engraftment is indicated by the plotted line while the letters indicate the type of engraftment (proportions of the various human hematopoietic lineages) detected in each model. The comparison between different models is not strictly quantitative because engraftment levels and lineages generated highly depend on the source of primary human cells and transplantation protocol. All *Rag2*$^{-/-}$*Il2Rgc*$^{-/-}$ strains are on the BALB/c background. NRG, *NOD-Rag1*$^{-/-}$*IL2Rg*$^{-/-}$; B2m, beta-2-microglobulin; NOD-Scid *B2m*$^{-/-}$.

as quiescence and cycling. Longer lifespan in humans greatly increases the risk of accumulating deleterious mutations, which imposes greater pressure on tumor suppression. As a result, human cells are more resistant to transformation (Hahn and Weinberg, 2002). Collectively, these considerations motivated the development of genetic tools and in vivo repopulation assays to study human stem cells (Dick, 2008). Although the focus on primary cells is highly relevant for human biology, the possibility always remains that some results may be artifacts of the surrogate in vitro or xenograft assay methods. While our review is focused on recent studies in humanized models, we will point out the frequent conservation and occasional key differences between mice and humans. Our view is that mouse and human cell models are complementary, and studies often need to be carried out in parallel.

Xenograft Models of Human Hematopoiesis

Inspired by the successful application of CFU-S assay to identify clonogenic progenitors in the mouse, investigation of human hematopoiesis first focused on colony-forming progenitors using in vitro CFU-C assay (Moore et al., 1973; Pike and Robinson, 1970). Using feeder layers from human peripheral blood (PB) to stimulate colony formation, Pike and Robinson demonstrated that rare cells in human BM generated CFU-Cs in agar. In the mouse, use of alternate feeder layers composed of adherent stromal cells revealed that primitive cell types, such as CFU-S, could be maintained in vitro (Dexter and Lajtha, 1974). By adapting these conditions, human CFU-Cs were continuously produced over weeks in culture (Gartner and Kaplan, 1980; Sutherland et al., 1989). The precursor cells giving rise to CFUs were referred to as long-term culture-initiating cells (LTC-ICs) and were positioned upstream of CFU-Cs. There were steady improvements in the LTC-IC assay with the use of cytokine-secreting stroma to augment multilineage differentiation and LTC-IC longevity (Sutherland et al., 1991). LTC-ICs are not a homogeneous population, but exhibit significant variation in their ability to sustain the cultures and maintain lympho-myeloid differentiation (Hao et al., 1996). While the LTC-IC assay represented a robust surrogate assay for multipotent cells, the relationship between LTC-ICs and HSCs, defined by repopulation potential, remained unclear, prompting the need for in vivo models for human cells.

Over 20 years have passed since primary human hematopoietic cells were first engrafted in immune-deficient mice (Figure 1). The first breakthrough in humanized mouse models was the

discovery of the severe combined immune-deficient (*Scid*) mouse lacking B and T cells (Fulop and Phillips, 1990; Bosma et al., 1983). Three independent approaches were initially used to engraft human hematopoietic cells in *Scid* mice. By infusing PB leukocytes (*Scid*-PBL model), Mosier et al. reconstituted human T and B cells capable of producing specific antibodies to tetanus toxin (Mosier et al., 1988, 1991). By surgically grafting human fetal tissues into *Scid* mice (*Scid*-hu model) and transplanting HLA-mismatched fetal liver cells, McCune et al. showed sustained production of donor human B and T cells indicative of stem/progenitor activity (McCune et al., 1988; Namikawa et al., 1988). These studies showed that human lymphocytes could survive and circulate in *Scid* mice, and be infected with HIV-1, establishing the first humanized AIDS models. Our group took a third approach that was based on human BMT and murine HSC assays. Lymphoid cells are long lived, while myeloid cells require rapid replenishment from progenitors and eventually from HSCs. Since no myeloid engraftment was observed in the *Scid*-hu model, and only limited numbers of macrophages were present in the *Scid*-PBL model, it remained unclear whether human HSCs could engraft and proliferate in immunodeficient mice. A formal demonstration of this requires serial assessment of myeloid cell potential after transplant. However, lack of cross-reactivity between the then newly discovered mouse and human myeloid growth factors was a concern. With this in mind, our group transplanted human BM cells intravenously into sublethally irradiated immune-deficient mice (*bg/nu/xid* and *Scid*) infused with human IL-3, GM-CSF, and SCF cytokines, and myeloid colony formation was tracked in the marrow of transplanted mice (Kamel-Reid and Dick, 1988; Lapidot et al., 1992). The results were clear: myeloid progenitors were generated even 4 months after transplant. Contemporaneous detection of B cells indicated that the engraftment was long term and multipotent, fulfilling two key criteria of HSCs. The cells that initiated engraftment in xenotransplants were operationally defined as *Scid*-repopulating cells (SRCs). This model provided a direct quantitative in vivo assay to measure human HSC activity and a means to undertake isolation of human HSCs.

The *Scid* model was limited; high levels of innate immune function and spontaneous emergence of B and T cells with age impeded human engraftment. To generate improved xenograft models, Shultz and colleagues backcrossed the *Scid* mutation onto nonobese diabetic (NOD) mice harboring defects in innate immunity. The resultant NOD-*scid* mice supported higher levels of human engraftment (Shultz et al., 1995). Interestingly, other backgrounds with the *Scid* mutation, such as nonobese resistant (NOR) or BALB/c, were nonsupportive (Shultz et al., 2007). Thus, background-specific genetic factors determine the capacity to engraft human cells. This conclusion was supported by studies in our laboratory showing that NOD, but not NOR, marrow stroma supported human LTC-IC (Takenaka et al., 2007). NOD mice are highly susceptible to spontaneous type 1 diabetes, and many insulin-dependent diabetes (*Idd*) loci were identified. Through a long positional cloning approach, the gene responsible for this supportive phenotype was identified to be *Sirpa* within the *Idd13* locus (Takenaka et al., 2007). Sirpa is a highly polymorphic transmembrane protein expressed on myeloid cells, and binding to its ligand CD47 inhibits phagocytosis. Human CD47 ubiquitously expressed on hematopoietic cells binds to NOD Sirpa with high affinity and induces host macrophage tolerance after transplant of human HSCs (Jaiswal et al., 2009; Takenaka et al., 2007). By contrast, NOR Sirpa does not bind human CD47, and NOD-*scid* mice with the NOR-*Sirpa* allele cannot be engrafted with human HSCs, establishing the importance of macrophages in HSC transplantation.

A major drawback to the NOD-*scid* model is the high incidence of thymic lymphoma, which prevents long-term studies (Shultz et al., 1995), and the fact that NK cells remain active and able to resist engraftment. To circumvent this problem, NOD-*scid* mice with either truncation (NOG) or a deletion in the IL-2R common γ chain (NSG), a critical component for IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 signaling, were developed (Ito et al., 2002; Shultz et al., 2005). The deletion of this gene in mice results in a complete loss of B, T, and NK cells. NSG mice support 5-fold higher $CD34^+$ cell engraftment compared with NOD-*scid* mice. Defects in cytokine signaling also prevent lymphomagenesis, permitting long-term analysis of human HSCs after transplant. Newer generations of mice are now being developed to better humanize the mice through the expression of human cytokines, such as thrombopoietin (TPO), IL-3, GM-CSF, and others that are not cross-reactive (Rongvaux et al., 2011; Willinger et al., 2011). Interestingly, sex-specific factors also affect human engraftment. Female NSG mice are 6-fold more sensitive at detecting single human HSCs (Notta et al., 2010). This observation suggests that yet undefined sex-specific factors, such as steroid hormones, can regulate human HSCs. The development of more and more robust xenograft models has enabled isolation and better characterization of human HSCs over the past 2 decades (Figure 1).

Isolation of Human HSCs

A major obstacle to studying HSC biology is that the cells are extremely rare. Only 1 in 10^6 cells in human BM is a transplantable HSC (Wang et al., 1997), requiring purification from the bulk of differentiated cells. Just as HSCs were discovered in the context of rescuing the effects of lethal doses of radiation, the activity of prospectively purified stem cell fractions can only be assayed by transplantation into conditioned hosts. To be defined as a stem cell, a cell must demonstrate durable self-renewal and differentiation into all cell types that compose the tissue. It should also do so at a clonal or single-cell level to exclude the possibility that a population that is homogeneous in terms of cell surface marker expression is still functionally heterogeneous and composed of multiple single-lineage precursors. These requirements present particular difficulties when testing human cells in xenografts. For example, in syngenic mouse experiments, long-term HSCs (LT-HSCs) have been historically defined as enabling repopulation beyond 12 weeks. Cells that generate all lineages but are only capable of transient engraftment are defined as short-term HSCs (ST-HSCs) or multipotent progenitors (MPPs). Even so, extended tracking for 6–8 months reveals so-called intermediate HSCs that extinguish between 3 and 6 months and are separable from both ST-HSCs and LT-HSCs (Benveniste et al., 2010). Defining the appropriate end-points for human cells in xenografts is more difficult. A 12-week period has been adopted by most investigators in the past. However, a longer period may be needed to distinguish between human transient and durable-reconstituting cells

(Glimm et al., 2001; Notta et al., 2011). In addition, production of different cell types in xenografts is temporally restricted. For instance, nucleated erythrocytes are found in the marrow 2–4 weeks after transplant, but they typically do not persist. On the other hand, thymic engraftment is not observed until 12 weeks after transplant, and peripheral T cells appear even later. At any given time point, not all lineages may be readily assayed, requiring careful kinetic assessment. These caveats notwithstanding, xenograft models can now be used to track self-renewal and multilineage output of single human cells over 8 months, fulfilling stringent criteria for HSCs (Notta et al., 2011).

Formative studies in stem cell biology have been carried out in mice. Mouse HSCs were first isolated as a lineage-negative (Lin^-), c-Kit^+, Sca-1^+ (LSK) population (Ikuta and Weissman, 1992; Spangrude et al., 1988). Within this subset, $CD34^-$ cells possess the unique capacity for long-term multilineage reconstitution and self-renewal (Osawa et al., 1996). About one in two or three $CD34^-$ LSK cells, alternatively defined by the $CD150^+CD48^-$ SLAM phenotype, possess LT-HSC activity (Kiel et al., 2005; Osawa et al., 1996). The ability to isolate purified HSCs has led to the detailed analysis of their transcriptional and epigenetic status (Ji et al., 2010; Ivanova et al., 2002; Ramalho-Santos et al., 2002). This detailed cellular picture of murine hematopoietic development (Figure 2A) combined with robust genetic approaches is beginning to unlock the molecular and biochemical pathways that underlie HSC function (Orkin and Zon, 2008).

As in the mouse, purification of human HSCs requires simultaneous detection of several independent cell surface markers. CD34, expressed on less than 5% of all blood cells, was the first marker found to enrich human HSCs and progenitors (Civin et al., 1984), and proven in numerous clinical HSCT studies over the past decade to mark HSCs (Kang et al., 2008; Vogel et al., 2000). Although some studies using xenograft assays suggest that, by analogy with the mouse, human $CD34^-$ HSCs might exist (Ishii et al., 2011; Bhatia et al., 1998), a simple calculation using reported frequencies indicates that >99% of human HSCs must be $CD34^+$. The lack of congruence of cell surface markers between mice and humans appears to be the rule rather than exception; for example, human HSCs express FLT3 receptor (Sitnicka et al., 2003) while mouse cells do not, and mouse HSCs express CD150 while human cells do not (Larochelle et al., 2011).

CD34 marks human HSCs as well as more differentiated progenitors, prompting a search for additional markers for fractionation. Using the *Scid*-hu model, Baum et al. identified CD90 (Thy1) as a stem cell marker (Baum et al., 1992). In combination with CD34, it demarcated a small population of $CD34^+Thy1^+$ cells that contained most multilineage capacity (Murray et al., 1995), and could mediate HSCT in breast cancer patients (Murray et al., 1995; Negrin et al., 2000). Further studies introduced CD45RA and CD38 as markers of more differentiated progenitors that negatively enrich for HSCs (Bhatia et al., 1997; Conneally et al., 1997; Lansdorp et al., 1990). Thus, a picture of human HSCs as $CD34^+CD38^-Thy1^+CD45RA^-$ (herein referred to as "$Thy1^+$") cells emerged over the past decade.

While a number of studies converged on the $Thy1^+$ phenotype for human HSCs, until recently little was known concerning its immediate progeny: the ST-HSC or MPP. Both of these cell types are defined by transient multilineage repopulation. The distinction between them is based on earlier mouse studies and conveys the idea that differentiation is a continuous process punctuated by many phenotypic states. A number of multipotent intermediates with varying degrees of self-renewal potential exist between long-term HSCs and the first lineage-committed progenitor. Identification of these intermediates would provide a molecular glimpse into the early events that coincide with the loss of self-renewal potential. The first hint of such a progenitor in humans came from transplants of $CD34^+CD38^{lo}$ umbilical cord blood (CB) cells into NOD-*Scid* mice that generated transient myelo-erythroid engraftment at 2 weeks (Mazurier et al., 2003). CD38 expression is gradually acquired by differentiating cells, and the $CD34^+CD38^{lo}$ fraction was still highly heterogeneous. In a more recent study, loss of Thy1 expression by $CD34+CD38^-CD45RA^-$ cells (referred to as "$Thy1^-$") was proposed to demarcate $Thy1^+$ HSCs from transiently-engrafting $Thy1^-$ MPPs (Majeti et al., 2007). However, $Thy1^-$ cells still mediated serial transfer, suggesting that this population was not completely resolved from HSCs. These studies pointed to the need to identify additional markers to separate HSCs from their nearest progeny that lack stem cell function.

Integrins mediate cell anchoring to the extracellular matrix (Raymond et al., 2009). As different cells utilize distinct combinations of integrins, they have been widely used to isolate stem cell populations from various normal and neoplastic tissues. For instance, integrin $\alpha 2$ (CD49b) differentially marks mouse long-term and intermediate-term HSCs (Benveniste et al., 2010), while integrin $\alpha 6$ (CD49f) marks normal mammary stem cells (Stingl et al., 1998), and malignant stem cells in glioblastoma (Lathia et al., 2010). We recently reported that CD49f is also expressed on ~50% of human $Thy1^+$ and ~25% of $Thy1^-$ cells, and when sorted fractions were assayed in vivo, HSC activity was restricted to the $CD49f^+$ cells in both fractions (Notta et al., 2011). By contrast, $Thy1^-CD49f^-$ cells mediate transient multilineage repopulation that peaks at 4 weeks and becomes undetectable after 16 weeks, reflective of MPPs. Transcriptional comparison of these closely related cell types provides an opening to begin to understand which changes in gene expression are associated with loss of stem cell function (Figure 2B).

Cellular and Molecular Mechanisms in Human HSCs

Molecular Regulators of Human HSCs

At the most fundamental level, of the two daughter cells produced after cell division of a multipotent cell, one, both, or neither may retain its identity as a stem cell. Till et al. were the first to describe these fate outcomes in probabilistic terms (Till et al., 1964). The molecular mechanisms that regulate this balance between self-renewal and differentiation are of primary interest in stem cell biology. These choices in cell fate are typically associated with changes in gene expression and are driven by transcription factors, although the initial changes often occur without de novo transcription and involve asymmetric distribution of fate determinants (Neumuller and Knoblich, 2009). Gene expression changes in stem cell differentiation are accompanied by, and are often preceded by, epigenetic changes in gene regulatory regions marking them as active, silent, or poised (Bernstein et al., 2006). It stands to reason that examination of global epigenetic and transcriptional differences between closely related stem-cell and non-stem-cell populations, such as HSCs and MPPs, would reveal a sequence of developmental events.

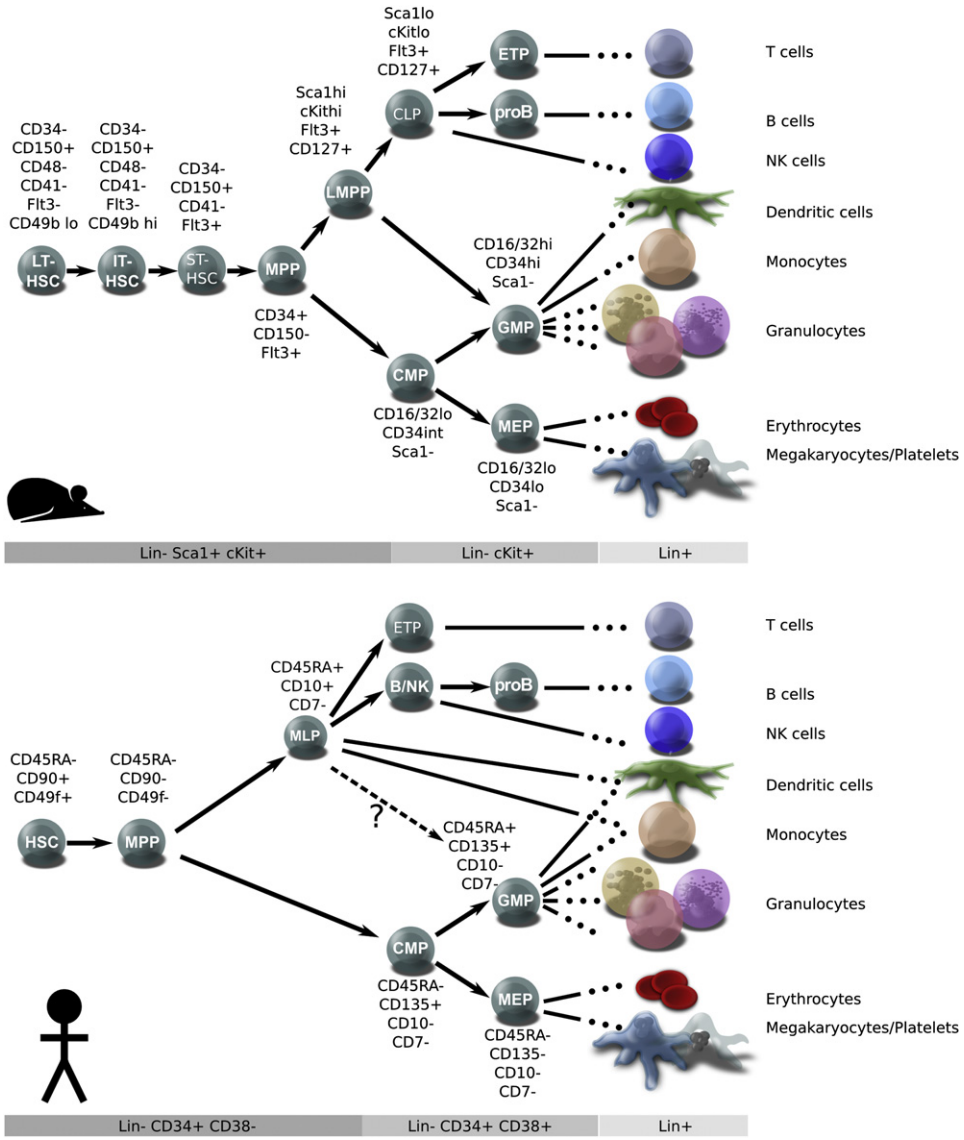

Figure 2. Current Models of Lineage Determination in the Adult Mouse and Human Hematopoietic Hierarchies

The major classes of stem and progenitor cells described in the text are defined by cell surface phenotypes, which are listed next to each population and in the gray bars below each schematic. Terminally differentiated cells are shown on the right, and inferred lineage relationships are depicted with arrows. In mice (A), HSCs can be separated into long-term (LT), intermediate-term (IT), and short-term (ST) classes based on the duration of repopulation. In humans (B), HSCs are defined by the expression of CD49f and other markers, but their heterogeneity has not been investigated. In mice, differentiation of HSCs gives rise to transiently engrafting multipotent progenitors (MPPs), and a series of immature lymphoid-biased progenitors (such as LMPPs) that undergo gradual lymphoid specification. In humans, MPPs can be identified by the loss of CD49f expression; however, only one population of immature lymphoid progenitors (MLPs) has been described. Both mice and humans have well-defined populations of myelo-erythroid progenitors: CMPs, GMPs, and MEPs. Lin: cocktail containing cell surface markers for all terminally differentiated populations (B cell; T cell; NK; dendritic cell, monocyte, granulocyte, megakaryocyte, and erythrocyte).

Such high-resolution maps that accompany cellular transitions have been generated for mouse hematopoiesis, and are being continuously refined (Ji et al., 2010). Until recently, only highly heterogeneous populations, based on the expression of CD34 and CD38, had been surveyed by gene expression profiling in human hematopoiesis (Georgantas et al., 2004). An earlier study also examined homologous genes with higher expression in LSK Rho^{lo} primitive mouse cells compared with Lin$^+$ cells. Of these, 39% were also more highly expressed in CB CD34$^+$CD38$^-$ primitive cells compared with human Lin$^+$ cells (Ivanova et al., 2002). This indicates conservation, but also specific differences between mouse and human HSC expression. Transcriptional comparison of CD49f$^+$ HSCs and Thy1$^-$CD49f$^-$ MPPs is yielding more precise information about the stem cell state. Several transcription factors are associated with the HSC state, including *ID* genes, *SOX8*, *SOX18*, and *NFIB*, while *MYC* and *IKZF1* are upregulated during differentiation into MPPs (Notta et al., 2011).

Regulators of stem cell function predicted by genomic approaches must be functionally validated. Steady improvements in xenograft models and lentiviral systems enable genetic

interrogation of primary human cells (Doulatov et al., 2009). A typical experimental design involves transduction of $CD34^+$ cells with lentiviruses designed for overexpression or shRNA-based gene silencing. Transduced cells are then sorted and injected into sublethally irradiated NSG mice, or seeded in surrogate stromal LTC-IC assays. To demonstrate an effect on self-renewal, serial transplantation is commonly employed. Below, we examine a small set of HSC regulators and cellular properties that have been best studied in human cells to date.

One of the most extensively studied HSC transcription factors is HoxB4. Retroviral transduction of mouse HSCs with *Hoxb4* expands stem cell numbers nearly 1,000-fold by activating symmetric self-renewal divisions with minimal incidence of leukemic transformation (Antonchuk et al., 2002). However, *HOXB4*-transduced human $CD34^+$ cells show a limited (2- to 4-fold) expansion of stem cell activity (Amsellem et al., 2003; Buske et al., 2002). HoxB4 also has the potential to direct mouse embryonic stem cells (ESCs) toward a hematopoietic fate, suggesting that it specifies HSCs in embryonic development (Kyba et al., 2002). By contrast, *HOXB4* does not confer repopulating potential on human ESC-derived hematopoietic cells (Wang et al., 2005). Thus, while HoxB4 may regulate some aspects of human HSC function, the data suggest that its function is not generally conserved in evolution.

Deletion of the Polycomb-group gene *Bmi1* leads to a lethal anemia in mice caused by the progressive loss of proliferative capacity of HSCs and progenitors (Lessard and Sauvageau, 2003). Similarly, knockdown of *BMI1* in human $CD34^+$ cells leads to loss of clonal potential (Rizo et al., 2009), while overexpression augments multilineage and serial replating potential (Rizo et al., 2008). Serial transplantation in NOD-*Scid* mice was used to show an effect of BMI1 on self-renewal. Thus, in this case, there is a correspondence between the findings in the mouse and human. Overexpression of BMI1 is found in myeloid leukemia and a wide range of solid tumors, and a number of studies highlight its role in tumor-initiating cells in hematopoietic, neural, and colon cancers (Schuringa and Vellenga, 2010). These findings suggest that the function of BMI1 is not only conserved across species, but also in many types of normal and malignant stem cells, posing it as a key target for therapeutic intervention.

The transcription factors *HLF* and Notch target *HES1* were initially identified by gene expression profiling of $CD34^+CD38^-$ cells from human fetal liver, CB, and adult marrow (Shojaei et al., 2005). Overexpression of *HES1* or *HLF* conferred increased repopulation potential; however, their activity in clonal assays or serial transfer was not tested (Shojaei et al., 2005). Canonical Notch signaling is initiated by binding of Jagged or Delta-like, which leads to proteolytic cleavage of the intracellular domain of Notch (ICN) by gamma-secretase and ICN translocation to the nucleus, where it participates in transcriptional activation (Pajcini et al., 2011). Interestingly, CCN3 (NOV), an extracellular modulator of Notch signaling, is also linked with human HSC function. NOV binds to the extracellular domain of Notch and increases expression of *Hes1* (Sakamoto et al., 2002). Loss of NOV impairs human LTC-IC activity and engraftment in NOD-*Scid* mice, whereas its enforced expression augments activity in both assays (Gupta et al., 2007). Reduction in levels of NOV is accompanied by decreased *HES1* expression, although it has not been shown that HES1 acts downstream of NOV. Collectively, these findings implicate Notch in maintaining human HSC function, which has been harnessed for clinical HSCT (see below). The role of the Notch pathway in murine HSCs has been widely studied. Recent evidence suggests that Notch does not have an obligate function in adult HSCs (Maillard et al., 2008), but constitutive activation of Notch can block differentiation and promote HSC expansion (Varnum-Finney et al., 2000). Thus, while it seems that Notch has a conserved role in mouse and human, additional studies should examine the requirement for Notch in human systems via loss-of-function or dominant-negative approaches.

Cellular Properties of Human HSCs

HSCs are known to reside in a quiescent state. Initial mouse BrdU incorporation label-retaining cell studies estimated that LT-HSCs divide once every 30–50 days (Cheshier et al., 1999; Kiel et al., 2007). An even more dormant subpopulation of LT-HSCs that only divides about five times in a mouse's lifetime was identified by two independent groups (Foudi et al., 2009; Wilson et al., 2008). Both of these studies genetically marked the chromatin using the H2B-GFP construct and followed this with very long chase periods. Cells that had not divided in more than 200 days were shown to contain most of the repopulation capacity upon transplantation. With such infrequent proliferative rates, these dormant HSCs, which represent only ~15% of the phenotypic LT-HSC pool, are thought not to contribute to daily production of hematopoietic cells, but to serve as a reservoir in case of injury. Because label-retaining cell studies cannot be carried out in humans, the existence of such rare and truly quiescent human HSCs cannot be assessed directly. Nonetheless, static measurements by flow cytometry indicate that the vast majority of human HSCs are in G_0. Studies relying on parameters measured in terminally differentiated cells, such as mean telomere length (Shepherd et al., 2004) or X chromosome inactivation ratios (Catlin et al., 2011), estimated that human HSCs divide every 175–350 days. Comparable approaches in mouse yielded estimates very similar to the ones obtained by the experimental label-retaining studies (Abkowitz et al., 1996). Thus, although human HSCs are predicted to replicate less frequently than their mouse counterparts, the number of divisions per lifetime appears to be roughly similar across species, supporting the idea that infrequent cycling is a protective mechanism limiting the accumulation of DNA damage due to replicative and oxidative stress.

Interestingly however, mouse and human HSCs appear to differ in their DNA damage response (DDR). Decreased sensitivity of mouse HSCs to cytotoxic agents, such as 5-FU, has been routinely used to enrich them from BM. The DDR response of quiescent mouse HSCs is biased to the prosurvival outcome due to p53-mediated activation of double-strand break (DSB) repair (Mohrin et al., 2010). While protecting HSCs from environmental insults, this mechanism leads to the accumulation of DNA damage in the pool of multipotent cells, increasing the chance of leukemic transformation. By contrast, human HSCs are actually sensitized to apoptosis after irradiation, sacrificing damaged HSCs in favor of maintaining genomic integrity (Milyavsky et al., 2010). This DDR is also p53 dependent, since loss of p53 compromises long-term HSC function and results in persistent DSB. Thus, an attractive hypothesis is that humans and mice have evolved different strategies to deal with DNA

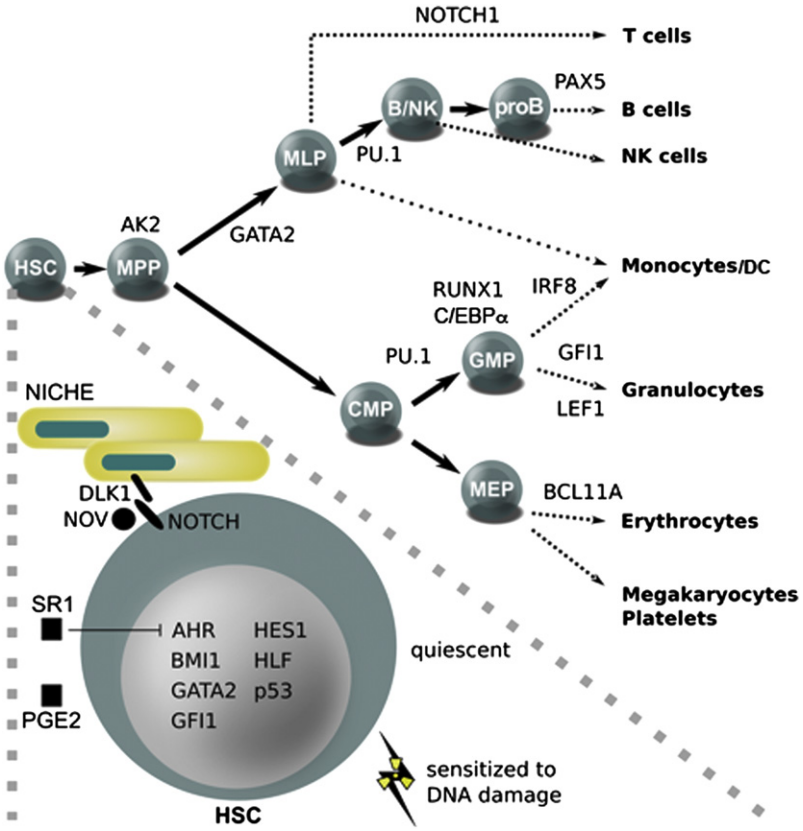

Figure 3. Molecules Implicated in Regulation of Human HSCs and Progenitors

Genes or pathways shown to control the function of human HSCs and early progenitors, using xenograft models and in vitro assays, or identified in patients with hematological malignancies, are overlaid on a scheme of the hematopoietic hierarchy from Figure 2B. The list is not exhaustive, but limited to the factors discussed in the text. Factors that have a demonstrated effect on human HSCs are shown on the left, including transcription factors and signaling pathways targeted for stem cell expansion. The details for each factor are presented in the text.

damage, reflecting different lifespans and age to reproductive maturity.

These select studies provide important parallels to mouse models and help identify promising pathways for therapeutic intervention (Figure 3). Important limitations remain to be overcome in the humanized models, including access to primary samples, improved xenograft models, and lentiviral expression systems for robust silencing and conditional transgene expression.

HSC-Based Therapeutics

Every year more than 30,000 patients with hematological malignancies receive high-dose chemotherapy followed by HSCT from BM, G-CSF mobilized PB, and CB. However, finding HLA-matched allogenic BM is still challenging. With the increased availability of banked samples, CB is becoming a more prominent source of cells for HSCT. However, successful engraftment is largely limited to pediatric cases because a single cord seldom yields enough HSCs for an adult (Brunstein and Wagner, 2006). Even modest HSC expansion would make CB transplants feasible for adults, greatly improving the number of potential donors. Moreover, it is well known that the time to neutrophil recovery (TNR), a major indicator of posttransplant mortality, is longer for CB than adult marrow (Brunstein and Wagner, 2006). Because neutrophil recovery depends on early-engrafting cells, and not LT-HSCs, the ability to expand these progenitors becomes equally critical. Traditional methods of culturing human $CD34^+$ cells in the presence of cytokines, such as SCF and TPO, showed marked effects on total cellularity due to their promotion of rapid proliferation; however, in most cases self renewal was not affected and HSC numbers did not increase (Miller et al., 2002). To establish that a method expands HSCs, rather than total cells or progenitors, requires measurement of HSC number before and after culture using repopulation assays. Until recently, few cytokine-based methods achieved this goal, and there is a clear need for novel approaches that significantly expand HSCs and/or lead to their improved engraftment following transplantation.

The search for compounds that modulate HSCs and early progenitor cells (HSPCs) can be based on rational targeting or random screening approaches, both of which have produced promising results. As discussed above, Notch signaling has a conserved role in both mouse and human hematopoiesis. Treatment of CB $CD34^+CD38^-$ cells with Notch ligand Delta-like 1 (DLK1) improves repopulating capacity in NOD-*Scid* mice without compromising differentiation potential (Ohishi et al., 2002). Bernstein and colleagues recently reported results of a small phase I trial in which ten patients were transplanted with DLK1-treated CB paired with an unmanipulated CB (Delaney et al., 2010). In this cohort of patients, median TNR was shortened significantly to 16 days compared with 26 days for unmanipulated CB. Short-term recovery was largely derived from the expanded CB. While these findings critically validate its utility in improving transplant outcome, the effect of Notch signaling on LT-HSCs remains in question. Long-term persistence of the expanded CB was only noted in two patients. Since the expanded CB lacks T cells, it is possible that its persistence is compromised by T cells from the unmanipulated CB. Alternatively, DLK1 may actually promote HSC differentiation, enhancing short-term output at the expense of durable repopulation. In this case, DLK1 treatment can only be used in paired transplants. Longer-term xenograft studies should help resolve these possibilities.

In a zebrafish screen for compounds that enhance the emergence of HSCs during embryogenesis, ~10% of positive hits targeted the prostaglandin pathway (North et al., 2007). Prostaglandins are small molecules that have diverse roles in smooth muscle contraction, blood clotting, inflammation, and pain. Treatment of zebrafish embryos with prostaglandin E2 (PGE2) augmented HSC formation. Unexpectedly, treatment of mouse

BM cells with PGE2 before transplant resulted in a 4-fold increase in the number of HSCs (North et al., 2007). This raises the question of why regulators of HSC specification in the embryo modulate adult HSC function. An attractive possibility is that these developmental pathways are shut off when HSCs enter quiescence around birth, but can be reactivated to promote symmetric self-renewal during injury and stress. Among these, the Wnt/β-catenin pathway is required for mesodermal development in embryogenesis, but is dispensable in adult hematopoiesis (Koch et al., 2008). However, genetic and chemical manipulation of Wnt signaling has been well documented to affect HSC expansion after transplant (Reya et al., 2003; Trowbridge et al., 2006). Interestingly, the regenerative effect of PGE2 is based on stabilization of β-catenin and activation of Wnt target genes linking these developmental pathways (Goessling et al., 2009). In a recent study, the effects of PGE2 were studied in a preclinical rhesus macaque model (Goessling et al., 2011). A 1 hr exposure of $CD34^+$ CB cells to PGE2 augmented HSC frequency in xenografts by ~2-fold, suggesting an effect on HSC homing rather than expansion. Because PGE2 has diverse, including many unwanted, biological effects, its safety is of foremost concern, and testing in the nonhuman primate model showed that brief exposure to PGE2 had no negative effects on long-term hematopoiesis.

Because the genetic basis for human stem cell function is poorly understood, rational approaches are often guided by model organism studies. These analyses should be complemented by unbiased screening in primary human cells to target critical yet unknown pathways. Differentiation of HSCs in culture is accompanied by loss of stem cell markers CD34, CD133, and Thy1. Compounds that delay or reverse this effect might affect expansion of HSCs. In a chemical screen, Boitano et al. identified a purine derivative termed StemRegenin-1 (SR1) among the compounds that maintain expression of CD34 and CD133 in culture (Boitano et al., 2010). Using a rigorous quantitative approach, they showed that a 3-week culture with SR1 increased the number of HSCs by 17-fold, which is to our knowledge the greatest reported expansion. SR1 is an antagonist of the aryl hydrocarbon receptor (AHR) and acts as a sensor for diverse xenobiotic compounds mediating steroid hormone signaling, inflammation, and T cell activation. A prototypic AHR ligand is dioxin, a ubiquitous environmental contaminant, which triggers immune suppression by AHR-mediated suppression of T cell maturation (Kerkvliet et al., 2002). Because AHR also binds polycyclic hydrocarbons, one interesting possibility is that it interferes with HSC maintenance in response to synthetic compounds in cell culture media and even plastic dishes. Although dioxin was known to decrease repopulation by LSK cells (Sakai et al., 2003), AHR was not identified as a key regulator of HSC function in mice, demonstrating the importance of unbiased approaches in human HSC studies. Several other compounds, including angiopoietin-like 5 (Angptl-5) and pleiotrophin, have been tested in the xenograft model (Himburg et al., 2010; Zhang et al., 2008). Because all preclinical models ultimately have serious limitations, expedited translation of promising candidates into small-scale clinical trials should help focus the field on the most relevant molecules and pathways for future investigation.

In addition to these more conventional directions, ESCs and induced pluripotent stem cells (iPSCs) represent a near-unlimited source of patient-specific HSCs. Recent studies have provided proof-of-principle that genetically corrected iPSCs can be used to treat hematological disorders (Hanna et al., 2007; Raya et al., 2009). However, there is at present no way to differentiate human ESCs/iPSCs into transplantable HSCs. In all of these translational approaches, development of more effective treatments requires better understanding of the biology and regulation of human HSCs and progenitors.

The Roadmap for Human Lineage Commitment

The Classical Model of Hematopoiesis

Blood cells belong to two fundamental branches: lymphoid and myeloid. The lymphoid branch consists of T, B, and NK cells, which carry out adaptive and innate immune responses. The myeloid lineage includes a number of distinct, fully differentiated, short-lived cell types including granulocytes (neutrophils, eosinophils, mast cells, and basophils), monocytes, erythrocytes, and megakaryocytes. As discussed above, multipotent HSCs reside at the apex of hematopoietic hierarchy and they are connected to mature cells by a complex roadmap of progenitor intermediates. Detailed analysis of this network can provide snapshots into ongoing developmental processes in which a single lineage program eventually becomes dominant while all others are repressed. A cellular roadmap that specifies lineage relationships between stem, progenitor, and mature cells is thus indispensable for a comprehensive view of the transcriptional and epigenetic mechanisms that control normal development.

The isolation of committed mouse progenitors of the myeloid and lymphoid lineages (CMPs and CLPs, respectively) using flow cytometric methods led to the formulation of the first comprehensive ''classical'' model of hematopoiesis (Akashi et al., 2000; Kondo et al., 1997; Reya et al., 2001). The first key postulate of this model is that loss of self-renewal capacity during differentiation precedes lineage commitment. This was inferred from the existence of MPPs, a progenitor type defined as LSK $CD34^+$ $Flt3^+$ that remains multipotent, but possesses only transient repopulation capacity (Adolfsson et al., 2001; Morrison et al., 1997). Another crucial postulate of the classical model is that the earliest commitment decision (downstream of MPPs) segregates lymphoid and myeloid lineages, inferred from the existence of CLPs and CMPs. Lastly, the classical model predicts that lineage decisions occur as stepwise bifurcations. The earliest myelo-lymphoid split gives rise to CMPs and CLPs and each of these undergo further commitment steps. CMPs give rise to GMPs, which become committed to the granulocyte-monocyte fate, and MEPs, which only produce erythroid and megakaryocyte (E-MK) cells. On the lymphoid side, CLPs give rise to B cell precursors and the earliest thymic progenitors (ETPs) committed to the T and NK lineages. The classical model is a simple yet powerful template for understanding blood development and interpreting the function of molecular regulators (Figure 2A).

Early Lymphoid Development in the Mouse

Using in vitro assays that support myeloid, B, and T cells, Kawamoto et al. meticulously cataloged the lineage output of single mouse fetal liver progenitors. They found that B, T, and erythroid fates were almost always coupled with myeloid potential, while a progenitor with restricted B and T cell output (i.e., CLPs) was

almost never observed (Kawamoto et al., 1999). This led the authors to propose an alternative "myeloid-based" model, in which lymphoid and myeloid fates remain coupled, instead of splitting early in differentiation (Kawamoto et al., 2010). One prediction of this model is the existence of progenitors with myelo-lymphoid, but not E-MK, potential. This was confirmed with the isolation of LSK CD34$^+$ Flt3^{hi} lymphoid-primed multipotent progenitors (LMPPs) from adult mouse marrow (Adolfsson et al., 2005). LMPPs display priming of lymphoid transcripts and mediate transient lympho-myeloid repopulation that displays lymphoid bias, but have a very low E-MK potential (Mansson et al., 2007). A number of early lymphoid progenitors varying in the degree of lineage bias and lymphoid commitment have been isolated in the past decade (Welner et al., 2008). The balance of available evidence supports the idea that lymphoid specification is not a single lineage bifurcation, but a gradual and possibly parallel process with many intermediate states. By contrast, myeloid development more closely adheres to the classical model. The main question has been whether erythrocytes and megakaryocytes are always derived from a CMP, or whether there are other possible branch points that give rise to MEPs—for instance, from an HSC or MPP, as previously suggested. Recent lineage tracing data with *Flk2*-Cre mice reported by two groups shows that the majority of erythrocytes and megakaryocytes are derived from a *Flk2*-positive multipotent progenitor (Boyer et al., 2011; Buza-Vidas et al., 2011). Because Flk2 is expressed by CMPs, but not fetal or adult HSCs, MPPs, and MEPs, it appears that most erythrocytes and megakaryocytes transition through a CMP stage, as predicted by the classical model. This asymmetry between the rapid myeloid and gradual lymphoid specification is undoubtedly reflected in the topology of the underlying transcription factor networks. It also has an epigenetic basis, because lymphoid development displays extensive methylation of myeloid promoters (Ji et al., 2010). Loss of Dnmt1, a key DNA methyltransferase in hematopoietic cells, impairs B and T cell development due to aberrant activation of myeloid genes (Broske et al., 2009). This suggests that mouse lymphoid development is critically dependent on epigenetic silencing of myeloid genes, and gradual shut-down of myeloid programs can explain the observation of multiple lymphoid intermediates with progressively restricted myeloid potentials.

If myeloid fates do persist in early lymphoid development, at what point do these programs become segregated to allow lymphoid commitment to actually take place? T cell development provides some intriguing clues. The thymus lacks self-renewing cells and is continuously seeded by progenitors from the marrow. Interestingly, immature ETPs, which reside in the thymic DN1 fraction, display both T cell and myeloid potential in OP9 cocultures and give rise to thymic myeloid cells in vivo (Bell and Bhandoola, 2008; Wada et al., 2008). Lineage tracing with *Rag* recombinase locus showed that about half of the ETPs and thymic Mac1$^+$Gr1$^+$ neutrophils were derived from *Rag*$^+$ progenitors and harbored rearranged TCR (Bell and Bhandoola, 2008). In an independent approach, DN1 cells gave rise to F4/80$^+$ macrophages in thymic lobe transplants (Wada et al., 2008). However, the ETPs had no detectable B cell potential. These studies suggest that the loss of myeloid potential is a relatively late event in T cell development, supporting myeloid-based models. However, contrary to this conclusion, *Il7ra* lineage tracing showed that while the majority of ETPs were derived from *Il7ra*$^+$ progenitors, most thymic myeloid cells were not (Schlenner et al., 2010). Still, approximately 20% of thymic neutrophils (compared to 2% of splenic neutrophils) originated from *Il7ra*$^+$ progenitors, which supports the myeloid potential of lymphoid progenitors, but questions the physiological relevance of this pathway.

Given the uncertainties in establishing precise lineage potential for any given population, particularly for human cells, we have proposed a broader term—multilymphoid progenitor (MLP)—to describe any progenitor that gives rise to all lymphoid lineages (B, T, and NK cells), but that may or may not have other (myeloid) potentials (Doulatov et al., 2010). Any B, T, and NK progenitor can be referred to as an MLP, whether or not its precise lineage output is ascertained. We will use this nomenclature below in reference to human hematopoiesis.

Clonal Assays to Define Developmental Potential

The conflicting observations from mouse studies raise a note of caution in interpreting results of in vitro assays. Single CLPs seeded in OP9 stromal cultures almost always generate myeloid colonies, but have minimal myeloid potential in the marrow and spleen after transplant (Richie Ehrlich et al., 2011). Thus, not surprisingly, in vitro potential does not always correlate with output in vivo. On the other hand, since progenitors lack the extensive proliferative potential of HSCs, it is impossible to assay single progenitors in vivo. Thus, in vitro systems have the advantages intrinsic to clonal assays.

Unlike for myelopoiesis, which can be studied in conventional CFU assays, known cytokines are insufficient to generate human B and T cells in vitro, complicating analysis of early human lymphoid development. Stromal lines established from irradiated mouse marrow, such as MS-5 and S17, provide robust support for human HSPCs (Collins and Dorshkind, 1987; Itoh et al., 1989). When cultured on MS-5 or S17 for 5–6 weeks in the absence of cytokines, HSPCs give rise to LTC-ICs. In shorter-term cultures, cytokines such as SCF promote proliferation and B cell development. By altering the cytokine cocktail to include SCF, TPO, IL-7, and IL-2, colonies composed of B, NK, and myeloid cells arise from single progenitors between 2 and 4 weeks. Concurrently, T cell development can be assayed on the OP9 stromal line expressing DLK1 (La Motte-Mohs et al., 2004).

To avoid misinterpretation, in vitro assays should fulfill certain minimum conditions. They should be carried out at a single-cell level, and support multilineage outputs to ascertain that these originate from a single multipotent cell. The output should also be efficient so that the fate of most cells can be accounted for, and lineage-committed controls should be used to show that the system does not alter lineage potential. Even if these criteria are fulfilled, lineage potential of isolated populations also needs to be confirmed by transplantation. However, given the possibility of independent routes of differentiation and transplant-related artifacts, this assay does not resolve whether a given population is a physiologically relevant intermediate. Lineage tracking can be used in mouse models to assess the contribution of any population provided its unique molecular characteristics, such as expression of *Il7ra*. In humans, patients with rare hematopoietic malignancies can provide insight into lineage potential of progenitors (see below).

A Model for Human Hematopoiesis

In applying the lessons learned in the mouse to human biology, one expects a general conservation; however, there is no a priori reason to believe that the same developmental strategies should necessarily be appropriate for dissimilar blood production requirements. For instance, human blood is neutrophil-rich, whereas mouse blood contains more lymphocytes. Despite this, the general assumption has been that the human hierarchy would be consistent with the classical model. Myeloid progenitors, CMPs, GMPs, and MEPs, were isolated based on the expression of IL-3 receptor α chain (CD123) or FLT3 (CD135), and CD45RA (Doulatov et al., 2010; Manz et al., 2002). Myeloid, but not erythroid, progenitors express CD123 and CD135, and the CMP to GMP transition is marked by acquisition of CD45RA. Single $CD135^+CD45RA^-$ CMPs produced all myeloid, but not lymphoid, lineages in vitro and after transplant. Thus, human myeloid development seems consistent with the classical model.

Mice and humans have evolved many distinct molecular mechanisms in immune development and response (Mestas and Hughes, 2004). An important example is the role of the γc chain and IL-7 in lymphoid development. Loss of γc in mice causes a combined B, T, and NK deficiency; of the cytokines that signal through γc, loss of IL-7 receptor (IL-7R) abolishes B and T cells. By contrast, γc deficiency in human SCID patients is characterized by T, but not B, cell deficiency (Noguchi et al., 1993); similarly, SCID patients with IL-7R mutations often have normal B cell counts (Puel et al., 1998). The practical consequences of this difference are that IL-7 is not sufficient to support human B cell development in culture (Billips et al., 1992; Prieyl and LeBien, 1996), and moreover, IL-7R delineates mouse, but not human, lymphoid progenitors. Instead, the search for the early lymphoid progenitors—which we refer to as MLPs—in human CB and adult BM has focused on CD7, the earliest T cell marker, and CD10, found on the earliest recognizable B cell precursors. Rare $CD7^+$ cells in the primitive $CD34^+CD38^-$ population were found to give rise to B and NK, but not myeloid or erythroid, cells in single-cell assays (Hao et al., 2001). A more recent study also reported robust T cell potential of this population (Hoebeke et al., 2007). However, $CD7^+$ cells are abundant in CB, but decline after birth, suggesting that most of them correspond to a wave of thymus-seeding progenitors (Haddad et al., 2006). While some of these may fulfill the criteria of MLPs, the question of which cells sustain lymphopoiesis in the adult remains.

In a foundational study using adult BM, $CD34^+CD10^+$ cells were shown to give rise to B, T, and NK cells, but not myeloid or erythroid progeny (Galy et al., 1995). This lineage output was partially confirmed by analysis of individual colonies on MS-5 stroma, although only rare cells had multilineage potential. Since CD10 and CD7 are also present on more mature B and T cells, it is not surprising that most $CD34^+CD10^+$ cells are more mature pro-B cells harboring DJ_H rearrangements (Rossi et al., 2003). More recently, another report showed that $CD34^+CD10^+$ cells depleted of $CD24^+$ pro-B cells are enriched for lymphoid potential in both CB and BM (Six et al., 2007). Importantly, these progenitors were found in circulation throughout life, and could be detected in the thymus. These reports suggested that human MLPs are largely lymphoid restricted and express an early B cell marker, CD10.

A more systematic analysis was required to investigate the relationship between these and other human progenitor classes reported to date. Using seven markers, we examined the lineage potential of neonate and adult progenitors using improved single-cell assays (Doulatov et al., 2010). Multilymphoid (B, T, and NK) potential was restricted to the $CD34^+CD38^-Thy1^{-/lo}CD45RA^+$ ($Thy1^-CD45RA^+$) compartment comprising just 1% of $CD34^+$ cells. Prior analysis of this population did not reveal in vivo repopulating activity, indicating that it was a more committed progenitor (Majeti et al., 2007). The question was whether early lymphoid progenitors in humans also retain myeloid programs, or if these are restricted during the initial differentiation decisions, as predicted by the classical model. Lymphoid colonies from single $Thy1^-CD45RA^+$ cells almost always contain myeloid cells, predominantly monocytes, macrophages, and dendritic cells. Moreover, $Thy1^-CD45RA^+$ cells transiently engraft NSG mice and generate both myelomonocytic and B cells, arguing that their myeloid potential is not an artifact of in vitro culture (Doulatov et al., 2010). Similar findings were reported using $Thy1^-CD45RA^+$ cells from adult BM (Goardon et al., 2011). They observed that many AML samples contain cells with the $Thy1^-CD45RA^+$ phenotype, which led them to investigate the developmental potential of these cells in normal BM. Indeed, these cells fulfilled the criteria of MLPs, giving rise to B, T, and NK cells, as well as myeloid lineages in vitro and in NSG mice, although multilineage output was not defined at the single-cell level. Myeloid output consisted of monocytes and granulocytes, although the latter was more modest, prompting them to conclude that these cells have lineage potential similar to that of LMPPs. Several differences in experimental design could account for the granulocytic potential in these studies compared with our own, including the age of marrow donors, since aged stem cells and progenitors display a prominent myeloid lineage bias (Rossi et al., 2008). Nevertheless, it is clear that human MLPs are not lymphoid restricted, and possess myeloid, but not erythroid and megakaryocytic, potential. Furthermore, they coexpress lymphoid-specific and myeloid-shared transcriptional programs consistent with their biological potential (see below). These findings allow us to propose a model for human hematopoiesis (Figure 2B).

Progenitor Origins of Dendritic Cells

Dendritic cells (DCs) are specialized antigen-presenting cells (APCs) that arise in the marrow and traffic to peripheral lymphoid organs (Shortman and Naik, 2007). Mice and humans harbor multiple DC subsets; however, there are many differences in their origin and function. Macrophages and DCs are monocytic cell types that are sometimes referred to as mononuclear phagocytes. It has been long hypothesized that mononuclear phagocytes are derived from a common progenitor (van Furth and Cohn, 1968). Indeed, $CX3CR1^+MCSFR^+$ macrophage-DC progenitors (MDPs) isolated from mouse marrow give rise to monocytes, macrophages, and steady-state spleen DCs, but have limited granulocytic potential (Fogg et al., 2006). These progenitors are phenotypically identical to GMPs, indicating that the GMP population can be separated into $CX3CR1^+$ MDPs and $CX3CR1^-$ GMPs and precursors of granulocytes. A corresponding progenitor in humans has not been identified,

but by analogy with the mouse, it might be defined by careful clonal analysis of the GMP fraction.

Mononuclear phagocytes also arise from MLPs/LMPPs, indicating that the molecular program that specifies these lineages remains active in the early stages of lymphoid development. Consistent with this, MLPs and GMPs share a significant portion of their transcriptome, coexpressing the transcription factors *SPI1* (PU.1), *MAF* (c-MAF), and *IRF8*, which are required for monocyte/DC development. Until recently it was thought that myeloid progenitors represented the main source of human DCs (Chicha et al., 2004). However, lymphoid progenitors were previously isolated as $CD7^+$ or $CD10^+$ $CD34^+CD38^+$ cells, which are already committed to T and B lineages. By contrast, more immature MLPs display a much greater DC potential in comparison with GMPs in vitro (Doulatov et al., 2010). Because MLPs can be isolated using magnetic columns from patient CB or PB, expanded, and matured under defined conditions into highly purified, mature DCs, they represent an attractive alternative to unfractionated $CD34^+$ progenitors for immune therapy applications. However, our study did not examine the origins of specialized APCs, such as plasmacytoid DCs and Langerhans cells, leaving the possibility that MLPs and GMPs might be biased to generate different DC subsets. Because tissue macrophages and DC subtypes can be studied in NSG xenografts (Cravens et al., 2005), future studies will examine the origins of these populations from human progenitors in vivo.

Global Molecular Programs in Human Hematopoiesis

Elucidation of the cellular hierarchy in human hematopoiesis provides a valuable resource for mapping the key regulatory networks that control blood differentiation and lineage commitment. This process involves global epigenetic and transcription changes that have been famously depicted in Waddington diagrams using a ''ball rolling down a hill'' analogy. In this model, initial small changes in protein levels are amplified by gene regulatory networks, setting off a cascade of gene expression states. This view raises two fundamental questions: what are the regulatory networks and epigenetic landmarks that shape this landscape and therefore control lineage commitment? And what are the forces acting on cells at each level, and how do these forces impact gene regulatory networks? Although a detailed picture of these mechanisms is emerging from mouse studies, comparison of transcriptional and epigenetic differences between different stages of differentiation in human hematopoiesis is just beginning (Figure 3).

Novershtern et al. compared transcriptional profiles of 38 human hematopoietic cell types, including precursors and mature cells (Novershtern et al., 2011). It is well known that components of regulatory networks are ''reused'' by different cell states. For instance, PU.1 is required for differentiation of monocytes and B cells (Carotta et al., 2010), while GFI1 is required for HSC self-renewal and granulocyte differentiation (van der Meer et al., 2010). To reveal this sharing, module analysis was used to search for coexpressed sets of genes. Indeed, most modules are shared between distinct cell states. For instance, ''HSC-persistent'' modules are shared between HSCs and progenitors. Global transcription factor usage was examined through analysis of *cis*-acting binding sites and expression-based prediction of regulatory interactions. Strikingly, of the 63 top module regulators, only 15 were previously associated with particular cell states in mouse studies (Novershtern et al., 2011). While this result may reflect the limitations of the computational methods, an alternative explanation is that transcription factor usage may differ somewhat between humans and mice. This does not mean that humans have evolved many entirely new regulatory mechanisms, but differences in state associations and timing of particular regulators could be widespread. For instance, globin switches in mammals are divergent: in the mouse, γ-globins are already repressed in definitive erythroid cells in the fetal liver, while in humans, γ-globin silencing only happens in the BM after birth. The explanation for this divergence lies in the fact that the expression of the full-length version of BCL11A, which is required for γ-globin repression in both species, is turned on at different times in mouse and human development (Sankaran et al., 2009). The study by Novershtern et al. should provide a rich source of candidates for functional validation in primary human cells. One shortcoming of the study is that it did not present a detailed coverage of the more immature progenitor and HSC compartments. Only myeloid progenitors were included, and HSCs were defined as $CD34^+CD38^-$. Thus, the upstream regulatory decisions that guide loss of HSC self-renewal, lineage priming, and specification are yet to be modeled.

Primary Myelo-Lymphoid Malignancies

Although experimental studies of human hematopoiesis require surrogate xenotransplantation or in vitro assays, clinical data on patients with hematological disorders provide a rich resource of regulatory mechanisms in hematopoiesis (Figure 3). Hematological disorders are typically grouped into nonmalignant disease, such as anemias, primary immunodeficiencies, and myelo-proliferative and myelo-dysplastic syndromes (MDS), or malignant leukemias of the myeloid (AML) or lymphoid (ALL) branches. The mutational landscape of these disorders is providing an increasingly focused view of the regulatory mechanisms that underlie specification of blood lineages. For instance, mutations in *GFI1*, *LEF1*, and *CEBPE* are found in severe congenital neutropenia (Klein, 2011); *IRF8* mutations occur in mononuclear phagocyte deficiency (Hambleton et al., 2011); and mutations in *CEBPA* and *RUNX1* are associated with familial MDS and AML (Owen et al., 2008), implicating these transcription factors as key regulators of myeloid development. Only a few have been studied experimentally in human cells. For example, the specific dominant-negative *CEBPA* mutation found in AML patients, which targets this conserved master regulator of granulocytic lineage, impairs myeloid differentiation in human, but not mouse, hematopoietic cells (Niebuhr et al., 2009; Schwieger et al., 2004). In lymphoid disease, *IKZF1* and *PAX5* deletions, master regulators of B cell development, occur in ~30% of B-ALL cases (Mullighan et al., 2009), whereas activating mutations of *NOTCH1*, the major T cell commitment pathway, are found in ~50% of T-ALL (Weng et al., 2004). A detailed description of these and other molecular pathways implicated in hematological disorders is the subject of a number of excellent reviews (Tenen, 2003). A common theme is that these diseases affect growth and differentiation of myeloid or lymphoid cells, but rarely both, implicating the underlying lesions in cells that are already lineage-committed. A fascinating, smaller group of disorders affect both myeloid and lymphoid branches, implying a role in multipotent cells. One such example

is reticular dysgenesis caused by mutations in adenylate kinase 2 (*AK2*), which plays a critical role in survival and metabolism of hematopoietic cells (Lagresle-Peyrou et al., 2009).

The classical example of biphenotypic disease is MLL leukemia. MLL is a mammalian homolog of *Trithorax*, which marks domains of active gene expression during development by H3K4 methylation. MLL translocates to over 50 partner genes and is found in over 50% of infant leukemias, which present as ALL, AML, or in some cases mixed-lineage diseases, with invariably poor prognosis (Krivtsov and Armstrong, 2007). Mouse models recapitulate many features of human disease, but primarily develop AML. By contrast, NOD-*Scid* mice transplanted with MLL-transduced human $CD34^+$ cells more often develop ALL (Barabe et al., 2007; Wei et al., 2008). Is this discrepancy due to species-specific differences? Monoclonal human MLL leukemias readily switch lineages between AML and ALL depending on microenvironmental cues (Barabe et al., 2007; Wei et al., 2008). Thus, the difference in disease spectrum in mouse and humanized models is likely explained by the different signaling in immune-competent and immune-deficient mice, the latter favoring B lymphopoiesis. The discovery of MLL fusions in leukemia has led to a dissection of its function in hematopoiesis. *Mll* null mice fail to specify HSCs during development (Ernst et al., 2004), and conditional models show that *Mll* is required for self-renewal of both fetal and adult HSCs (Jude et al., 2007). Thus, the translation between mouse and human models of malignant and normal hematopoiesis provides a powerful paradigm for understanding biological systems.

Another interesting example of multilineage disease comes from the recent reported description of a heterogeneous group of patients with a combined DC, monocyte, and B- and NK-lymphoid (DCML) deficiency (also referred to as "monoMAC") (Bigley et al., 2011; Vinh et al., 2010). Many patients exhibit myelodysplastic features and predisposition to leukemia, but granulocyte, erythrocyte, platelet, and T cell counts at diagnosis are largely normal. The myeloid and lymphoid lineages affected in DCML are reminiscent of the lineage output of MLP. Flow cytometric analysis of $CD34^+$ cells in the BM of DCML patients revealed a complete absence of MLPs, as well as the downstream $CD38^+CD10^+$ B/NK progenitors, and a marked reduction in GMPs (Bigley et al., 2011). The HSC and MPP compartments were unaffected, indicating a block in MPP to MLP differentiation. This suggests that the MLP is an obligate intermediate in human B and NK development. In addition, monocyte and DC differentiation proceed through MLPs, consistent with experimental models. The alternative model in which these lineages are derived solely from myeloid progenitors is not consistent with the clinical findings, since GMPs were reduced but not absent. Furthermore, the GMPs remained functional as granulocyte numbers remained within normal ranges. Interestingly, T cell numbers in these patients were normal, despite the lack of MLPs. Since T cells are long lived, this could be due to their generation from an earlier time point when MLPs might still have been present. Alternatively, this result is also consistent with the recent demonstration that multiple progenitor types can contribute to thymopoiesis in mice (Saran et al., 2010). Exome sequencing in DCML patients revealed distinct allelic loss-of-function mutations in the *GATA2* gene (Dickinson et al., 2011; Hsu et al., 2011). Gata2 is required for the initiation of definitive hematopoiesis in mice, and maintains the proliferative output of stem and progenitor cells (Tsai and Orkin, 1997). Notably, unlike DCML, blood cell numbers in $Gata2^{+/-}$ mice are largely unperturbed, despite reduced proliferation of immature progenitors (Rodrigues et al., 2005). This finding indicates that GATA2 haploinsufficiency has different effects in humans and mice, suggesting that it is a key regulator of MLPs and early lymphoid fate in humans. Improved detection and genomic sequencing of patients with rare primary immunodeficiency will create a rich resource of pathways for detailed interrogation in xenograft models.

Conclusions

Advances in our ability to investigate human hematopoiesis from a cellular and molecular viewpoint now offer the possibility of complementing the information from murine models of normal hematopoietic development with parallel studies in primary human cells. This development is especially applicable for studies aimed at preclinical testing of new therapies for expansion of normal HSCs and eradication of their leukemic counterparts using xenograft models. Recent successes with DLK1, SR1, and PGE2 in improving transplant outcomes attest to the relevance of this strategy. Single HSCs, which can now be isolated with remarkable purity from human blood and marrow, stably regenerate the blood hierarchy in transplanted immune-deficient mice. These purified HSCs can be used in chemical screens for compounds that mediate expansion, survival, or other effects. More globally, the roadmap of human hematopoiesis outlines the major cellular steps during HSC differentiation, and serves as a template for understanding the molecular basis of developmental transitions. It therefore opens the way for comprehensive global profiling to identify candidate regulators for validation by gene targeting, contributing to an understanding of molecular networks that underlie fate decisions. Our focus here was on normal hematopoiesis, and knowledge of normal development provides a much needed context to understand the impact of molecular aberrations that are now being identified at an increased pace in human leukemias. Defects in the molecular components that control hematopoiesis severely perturb normal development and lie at the root of hematological malignancies. Indeed, the development of xenograft models and studies on normal HSCs led to the identification of leukemic stem cells (LSCs) over a decade ago (explored in Dick, 2008), and recent studies from our group showed that LSCs and HSCs share gene expression programs (Eppert et al., 2011). Continued investigation of normal hematopoiesis using both mouse and human systems is critical to gain a deeper insight into malignancy.

ACKNOWLEDGMENTS

This work was supported by Canadian Institutes for Health Research (CIHR) studentships (to F.N. and S.D.), the Swiss National Science Foundation (E.L.), and Roche fellowships (E.L.). Grants were from the CIHR, Canadian Cancer Society Research Institute, Terry Fox Foundation, Genome Canada through the Ontario Genomics Institute, Ontario Institute for Cancer Research with funds from the province of Ontario, the Cancer Stem Cell Consortium with funding from the Government of Canada through Genome Canada, the Ontario Genomics Institute (OGI-047), and a Canada Research Chair. This research was funded in part by the Ontario Ministry of Health and Long Term Care (OMOHLTC). The views expressed do not necessarily reflect those

of the OMOHLTC. Due to length restrictions, we apologize to all the colleagues whose contributions we could not properly acknowledge in this review.

REFERENCES

Abkowitz, J.L., Catlin, S.N., and Guttorp, P. (1996). Evidence that hematopoiesis may be a stochastic process in vivo. Nat. Med. *2*, 190–197.

Adolfsson, J., Borge, O.J., Bryder, D., Theilgaard-Monch, K., Astrand-Grundstrom, I., Sitnicka, E., Sasaki, Y., and Jacobsen, S.E. (2001). Upregulation of Flt3 expression within the bone marrow Lin(-)Sca1(+)c-kit(+) stem cell compartment is accompanied by loss of self-renewal capacity. Immunity *15*, 659–669.

Adolfsson, J., Mansson, R., Buza-Vidas, N., Hultquist, A., Liuba, K., Jensen, C.T., Bryder, D., Yang, L., Borge, O.J., Thoren, L.A., et al. (2005). Identification of Flt3+ lympho-myeloid stem cells lacking erythro-megakaryocytic potential a revised road map for adult blood lineage commitment. Cell *121*, 295–306.

Akashi, K., Traver, D., Miyamoto, T., and Weissman, I.L. (2000). A clonogenic common myeloid progenitor that gives rise to all myeloid lineages. Nature *404*, 193–197.

Amsellem, S., Pflumio, F., Bardinet, D., Izac, B., Charneau, P., Romeo, P.H., Dubart-Kupperschmitt, A., and Fichelson, S. (2003). Ex vivo expansion of human hematopoietic stem cells by direct delivery of the HOXB4 homeoprotein. Nat. Med. *9*, 1423–1427.

Antonchuk, J., Sauvageau, G., and Humphries, R.K. (2002). HOXB4-induced expansion of adult hematopoietic stem cells ex vivo. Cell *109*, 39–45.

Barabe, F., Kennedy, J.A., Hope, K.J., and Dick, J.E. (2007). Modeling the initiation and progression of human acute leukemia in mice. Science *316*, 600–604.

Baum, C.M., Weissman, I.L., Tsukamoto, A.S., Buckle, A.M., and Peault, B. (1992). Isolation of a candidate human hematopoietic stem-cell population. Proc. Natl. Acad. Sci. USA *89*, 2804–2808.

Becker, A.J., McCulloch, E.A., and Till, J.E. (1963). Cytological Demonstration of the Clonal Nature of Spleen Colonies Derived from Transplanted Mouse Marrow Cells. Nature *197*, 452–454.

Bell, J.J., and Bhandoola, A. (2008). The earliest thymic progenitors for T cells possess myeloid lineage potential. Nature *452*, 764–767.

Benveniste, P., Frelin, C., Janmohamed, S., Barbara, M., Herrington, R., Hyam, D., and Iscove, N.N. (2010). Intermediate-term hematopoietic stem cells with extended but time-limited reconstitution potential. Cell Stem Cell *6*, 48–58.

Bernstein, B.E., Mikkelsen, T.S., Xie, X., Kamal, M., Huebert, D.J., Cuff, J., Fry, B., Meissner, A., Wernig, M., Plath, K., et al. (2006). A bivalent chromatin structure marks key developmental genes in embryonic stem cells. Cell *125*, 315–326.

Bhatia, M., Wang, J.C.Y., Kapp, U., Bonnet, D., and Dick, J.E. (1997). Purification of primitive human hematopoietic cells capable of repopulating immune-deficient mice. Proc. Natl. Acad. Sci. USA *94*, 5320–5325.

Bhatia, M., Bonnet, D., Murdoch, B., Gan, O.I., and Dick, J.E. (1998). A newly discovered class of human hematopoietic cells with SCID- repopulating activity. Nat. Med. *4*, 1038–1045.

Bigley, V., Haniffa, M., Doulatov, S., Wang, X.N., Dickinson, R., McGovern, N., Jardine, L., Pagan, S., Dimmick, I., Chua, I., et al. (2011). The human syndrome of dendritic cell, monocyte, B and NK lymphoid deficiency. J. Exp. Med. *208*, 227–234.

Billips, L.G., Petitte, D., Dorshkind, K., Narayanan, R., Chiu, C.P., and Landreth, K.S. (1992). Differential roles of stromal cells, interleukin-7, and kit-ligand in the regulation of B lymphopoiesis. Blood *79*, 1185–1192.

Boitano, A.E., Wang, J., Romeo, R., Bouchez, L.C., Parker, A.E., Sutton, S.E., Walker, J.R., Flaveny, C.A., Perdew, G.H., Denison, M.S., et al. (2010). Aryl hydrocarbon receptor antagonists promote the expansion of human hematopoietic stem cells. Science *329*, 1345–1348.

Bosma, G.C., Custer, R.P., and Bosma, M.J. (1983). A severe combined immunodeficiency mutation in the mouse. Nature *301*, 527–530.

Boyer, S.W., Schroeder, A.V., Smith-Berdan, S., and Forsberg, E.C. (2011). All hematopoietic cells develop from hematopoietic stem cells through Flk2/Flt3-positive progenitor cells. Cell Stem Cell *9*, 64–73.

Broske, A.M., Vockentanz, L., Kharazi, S., Huska, M.R., Mancini, E., Scheller, M., Kuhl, C., Enns, A., Prinz, M., Jaenisch, R., et al. (2009). DNA methylation protects hematopoietic stem cell multipotency from myeloerythroid restriction. Nat. Genet. *41*, 1207–1215.

Brunstein, C.G., and Wagner, J.E. (2006). Umbilical cord blood transplantation and banking. Annu. Rev. Med. *57*, 403–417.

Buske, C., Feuring-Buske, M., Abramovich, C., Spiekermann, K., Eaves, C.J., Coulombel, L., Sauvageau, G., Hogge, D.E., and Humphries, R.K. (2002). Deregulated expression of HOXB4 enhances the primitive growth activity of human hematopoietic cells. Blood *100*, 862–868.

Buza-Vidas, N., Woll, P., Hultquist, A., Duarte, S., Lutteropp, M., Bouriez-Jones, T., Ferry, H., Luc, S., and Jacobsen, S.E. (2011). FLT3 expression initiates in fully multipotent mouse hematopoietic progenitor cells. Blood *118*, 1544–1548.

Carotta, S., Wu, L., and Nutt, S.L. (2010). Surprising new roles for PU.1 in the adaptive immune response. Immunol. Rev. *238*, 63–75.

Catlin, S.N., Busque, L., Gale, R.E., Guttorp, P., and Abkowitz, J.L. (2011). The replication rate of human hematopoietic stem cells in vivo. Blood *117*, 4460–4466.

Cheshier, S.H., Morrison, S.J., Liao, X., and Weissman, I.L. (1999). In vivo proliferation and cell cycle kinetics of long-term self-renewing hematopoietic stem cells. Proc. Natl. Acad. Sci. USA *96*, 3120–3125.

Chicha, L., Jarrossay, D., and Manz, M.G. (2004). Clonal type I interferon-producing and dendritic cell precursors are contained in both human lymphoid and myeloid progenitor populations. J. Exp. Med. *200*, 1519–1524.

Civin, C.I., Strauss, L.C., Brovall, C., Fackler, M.J., Schwartz, J.F., and Shaper, J.H. (1984). Antigenic analysis of hematopoiesis. III. A hematopoietic progenitor cell surface antigen defined by a monoclonal antibody raised against KG-1a cells. J. Immunol. *133*, 157–165.

Collins, L.S., and Dorshkind, K. (1987). A stromal cell line from myeloid long-term bone marrow cultures can support myelopoiesis and B lymphopoiesis. J. Immunol. *138*, 1082–1087.

Conneally, E., Cashman, J., Petzer, A., and Eaves, C. (1997). Expansion in vitro of transplantable human cord blood stem cells demonstrated using a quantitative assay of their lympho-myeloid repopulating activity in nonobese diabetic-scid/scid mice. Proc. Natl. Acad. Sci. USA *94*, 9836–9841.

Cravens, P.D., Melkus, M.W., Padgett-Thomas, A., Islas-Ohlmayer, M., Del, P.M.M., and Garcia, J.V. (2005). Development and activation of human dendritic cells in vivo in a xenograft model of human hematopoiesis. Stem Cells *23*, 264–278.

Delaney, C., Heimfeld, S., Brashem-Stein, C., Voorhies, H., Manger, R.L., and Bernstein, I.D. (2010). Notch-mediated expansion of human cord blood progenitor cells capable of rapid myeloid reconstitution. Nat. Med. *16*, 232–236.

Dexter, T.M., and Lajtha, L.G. (1974). Proliferation of haemopoietic stem cells in vitro. Br. J. Haematol. *28*, 525–530.

Dick, J.E. (2008). Stem cell concepts renew cancer research. Blood *112*, 4793–4807.

Dickinson, R.E., Griffin, H., Bigley, V., Reynard, L.N., Hussain, R., Haniffa, M., Lakey, J.H., Rahman, T., Wang, X.N., McGovern, N., et al. (2011). Exome sequencing identifies GATA-2 mutation as the cause of dendritic cell, monocyte, B and NK lymphoid deficiency. Blood *118*, 2656–2658.

Doulatov, S., Notta, F., Rice, K.L., Howell, L., Zelent, A., Licht, J.D., and Dick, J.E. (2009). PLZF is a regulator of homeostatic and cytokine-induced myeloid development. Genes Dev. *23*, 2076–2087.

Doulatov, S., Notta, F., Eppert, K., Nguyen, L.T., Ohashi, P.S., and Dick, J.E. (2010). Revised map of the human progenitor hierarchy shows the origin of macrophages and dendritic cells in early lymphoid development. Nat. Immunol. *11*, 585–593.

Eppert, K., Takenaka, K., Lechman, E.R., Waldron, L., Nilsson, B., van Galen, P., Metzeler, K.H., Poeppl, A., Ling, V., Beyene, J., et al. (2011). Stem cell gene

expression programs influence clinical outcome in human leukemia. Nat. Med. *17*, 1086–1093.

Ernst, P., Fisher, J.K., Avery, W., Wade, S., Foy, D., and Korsmeyer, S.J. (2004). Definitive hematopoiesis requires the mixed-lineage leukemia gene. Dev. Cell *6*, 437–443.

Fogg, D.K., Sibon, C., Miled, C., Jung, S., Aucouturier, P., Littman, D.R., Cumano, A., and Geissmann, F. (2006). A clonogenic bone marrow progenitor specific for macrophages and dendritic cells. Science *311*, 83–87.

Foudi, A., Hochedlinger, K., Van Buren, D., Schindler, J.W., Jaenisch, R., Carey, V., and Hock, H. (2009). Analysis of histone 2B-GFP retention reveals slowly cycling hematopoietic stem cells. Nat. Biotechnol. *27*, 84–90.

Fulop, G.M., and Phillips, R.A. (1990). The scid mutation in mice causes a general defect in DNA repair. Nature *347*, 479–482.

Galy, A., Travis, M., Cen, D., and Chen, B. (1995). Human T, B, natural killer, and dendritic cells arise from a common bone marrow progenitor cell subset. Immunity *3*, 459–473.

Gartner, S., and Kaplan, H.S. (1980). Long-term culture of human bone marrow cells. Proc. Natl. Acad. Sci. USA *77*, 4756–4759.

Georgantas, R.W., 3rd, Tanadve, V., Malehorn, M., Heimfeld, S., Chen, C., Carr, L., Martinez-Murillo, F., Riggins, G., Kowalski, J., and Civin, C.I. (2004). Microarray and serial analysis of gene expression analyses identify known and novel transcripts overexpressed in hematopoietic stem cells. Cancer Res. *64*, 4434–4441.

Glimm, H., Eisterer, W., Lee, K., Cashman, J., Holyoake, T.L., Nicolini, F., Shultz, L.D., von Kalle, C., and Eaves, C.J. (2001). Previously undetected human hematopoietic cell populations with short-term repopulating activity selectively engraft NOD/SCID-beta2 microglobulin-null mice. J. Clin. Invest. *107*, 199–206.

Goardon, N., Marchi, E., Atzberger, A., Quek, L., Schuh, A., Soneji, S., Woll, P., Mead, A., Alford, K.A., Rout, R., et al. (2011). Coexistence of LMPP-like and GMP-like leukemia stem cells in acute myeloid leukemia. Cancer Cell *19*, 138–152.

Goessling, W., North, T.E., Loewer, S., Lord, A.M., Lee, S., Stoick-Cooper, C.L., Weidinger, G., Puder, M., Daley, G.Q., Moon, R.T., et al. (2009). Genetic interaction of PGE2 and Wnt signaling regulates developmental specification of stem cells and regeneration. Cell *136*, 1136–1147.

Goessling, W., Allen, R.S., Guan, X., Jin, P., Uchida, N., Dovey, M., Harris, J.M., Metzger, M.E., Bonifacino, A.C., Stroncek, D., et al. (2011). Prostaglandin E2 enhances human cord blood stem cell xenotransplants and shows long-term safety in preclinical nonhuman primate transplant models. Cell Stem Cell *8*, 445–458.

Gupta, R., Hong, D., Iborra, F., Sarno, S., and Enver, T. (2007). NOV (CCN3) functions as a regulator of human hematopoietic stem or progenitor cells. Science *316*, 590–593.

Haddad, R., Guimiot, F., Six, E., Jourquin, F., Setterblad, N., Kahn, E., Yagello, M., Schiffer, C., Andre-Schmutz, I., Cavazzana-Calvo, M., et al. (2006). Dynamics of thymus-colonizing cells during human development. Immunity *24*, 217–230.

Hahn, W.C., and Weinberg, R.A. (2002). Rules for making human tumor cells. N. Engl. J. Med. *347*, 1593–1603.

Hambleton, S., Salem, S., Bustamante, J., Bigley, V., Boisson-Dupuis, S., Azevedo, J., Fortin, A., Haniffa, M., Ceron-Gutierrez, L., Bacon, C.M., et al. (2011). IRF8 mutations and human dendritic-cell immunodeficiency. N. Engl. J. Med. *365*, 127–138.

Hanna, J., Wernig, M., Markoulaki, S., Sun, C.W., Meissner, A., Cassady, J.P., Beard, C., Brambrink, T., Wu, L.C., Townes, T.M., et al. (2007). Treatment of sickle cell anemia mouse model with iPS cells generated from autologous skin. Science *318*, 1920–1923.

Hao, Q.L., Thiemann, F.T., Petersen, D., Smogorzewska, E.M., and Crooks, G.M. (1996). Extended long-term culture reveals a highly quiescent and primitive human hematopoietic progenitor population. Blood *88*, 3306–3313.

Hao, Q.L., Zhu, J., Price, M.A., Payne, K.J., Barsky, L.W., and Crooks, G.M. (2001). Identification of a novel, human multilymphoid progenitor in cord blood. Blood *97*, 3683–3690.

Himburg, H.A., Muramoto, G.G., Daher, P., Meadows, S.K., Russell, J.L., Doan, P., Chi, J.T., Salter, A.B., Lento, W.E., Reya, T., et al. (2010). Pleiotrophin regulates the expansion and regeneration of hematopoietic stem cells. Nat. Med. *16*, 475–482.

Hoebeke, I., De Smedt, M., Stolz, F., Pike-Overzet, K., Staal, F.J., Plum, J., and Leclercq, G. (2007). T-, B- and NK-lymphoid, but not myeloid cells arise from human CD34(+)CD38(-)CD7(+) common lymphoid progenitors expressing lymphoid-specific genes. Leukemia *21*, 311–319.

Hsu, A.P., Sampaio, E.P., Khan, J., Calvo, K.R., Lemieux, J.E., Patel, S.Y., Frucht, D.M., Vinh, D.C., Auth, R.D., Freeman, A.F., et al. (2011). Mutations in GATA2 are associated with the autosomal dominant and sporadic monocytopenia and mycobacterial infection (MonoMAC) syndrome. Blood *118*, 2653–2655.

Ikuta, K., and Weissman, I.L. (1992). Evidence that hematopoietic stem cells express mouse c-kit but do not depend on steel factor for their generation. Proc. Natl. Acad. Sci. USA *89*, 1502–1506.

Ishii, M., Matsuoka, Y., Sasaki, Y., Nakatsuka, R., Takahashi, M., Nakamoto, T., Yasuda, K., Matsui, K., Asano, H., Uemura, Y., et al. (2011). Development of a high-resolution purification method for precise functional characterization of primitive human cord blood-derived CD34-negative SCID-repopulating cells. Exp. Hematol *39*, 203–213, e201.

Ito, M., Hiramatsu, H., Kobayashi, K., Suzue, K., Kawahata, M., Hioki, K., Ueyama, Y., Koyanagi, Y., Sugamura, K., Tsuji, K., et al. (2002). NOD/SCID/gamma(c)(null) mouse: an excellent recipient mouse model for engraftment of human cells. Blood *100*, 3175–3182.

Itoh, K., Tezuka, H., Sakoda, H., Konno, M., Nagata, K., Uchiyama, T., Uchino, H., and Mori, K.J. (1989). Reproducible establishment of hemopoietic supportive stromal cell lines from murine bone marrow. Exp. Hematol. *17*, 145–153.

Ivanova, N.B., Dimos, J.T., Schaniel, C., Hackney, J.A., Moore, K.A., and Lemischka, I.R. (2002). A stem cell molecular signature. Science *298*, 601–604.

Jaiswal, S., Jamieson, C.H., Pang, W.W., Park, C.Y., Chao, M.P., Majeti, R., Traver, D., van Rooijen, N., and Weissman, I.L. (2009). CD47 is upregulated on circulating hematopoietic stem cells and leukemia cells to avoid phagocytosis. Cell *138*, 271–285.

Ji, H., Ehrlich, L.I., Seita, J., Murakami, P., Doi, A., Lindau, P., Lee, H., Aryee, M.J., Irizarry, R.A., Kim, K., et al. (2010). Comprehensive methylome map of lineage commitment from haematopoietic progenitors. Nature *467*, 338–342.

Jude, C.D., Climer, L., Xu, D., Artinger, E., Fisher, J.K., and Ernst, P. (2007). Unique and independent roles for MLL in adult hematopoietic stem cells and progenitors. Cell Stem Cell *1*, 324–337.

Kamel-Reid, S., and Dick, J.E. (1988). Engraftment of immune-deficient mice with human hematopoietic stem cells. Science *242*, 1706–1709.

Kang, Y., Chao, N.J., and Aversa, F. (2008). Unmanipulated or CD34 selected haplotype mismatched transplants. Curr. Opin. Hematol. *15*, 561–567.

Kawamoto, H., Ohmura, K., Fujimoto, S., and Katsura, Y. (1999). Emergence of T cell progenitors without B cell or myeloid differentiation potential at the earliest stage of hematopoiesis in the murine fetal liver. J. Immunol. *162*, 2725–2731.

Kawamoto, H., Ikawa, T., Masuda, K., Wada, H., and Katsura, Y. (2010). A map for lineage restriction of progenitors during hematopoiesis: the essence of the myeloid-based model. Immunol. Rev. *238*, 23–36.

Kerkvliet, N.I., Shepherd, D.M., and Baecher-Steppan, L. (2002). T lymphocytes are direct, aryl hydrocarbon receptor (AhR)-dependent targets of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD): AhR expression in both CD4+ and CD8+ T cells is necessary for full suppression of a cytotoxic T lymphocyte response by TCDD. Toxicol. Appl. Pharmacol. *185*, 146–152.

Kiel, M.J., Yilmaz, O.H., Iwashita, T., Terhorst, C., and Morrison, S.J. (2005). SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. Cell *121*, 1109–1121.

Kiel, M.J., He, S., Ashkenazi, R., Gentry, S.N., Teta, M., Kushner, J.A., Jackson, T.L., and Morrison, S.J. (2007). Haematopoietic stem cells do not asymmetrically segregate chromosomes or retain BrdU. Nature *449*, 238–242.

Klein, C. (2011). Genetic defects in severe congenital neutropenia: emerging insights into life and death of human neutrophil granulocytes. Annu. Rev. Immunol. *29*, 399–413.

Koch, U., Wilson, A., Cobas, M., Kemler, R., Macdonald, H.R., and Radtke, F. (2008). Simultaneous loss of beta- and gamma-catenin does not perturb hematopoiesis or lymphopoiesis. Blood *111*, 160–164.

Kondo, M., Weissman, I.L., and Akashi, K. (1997). Identification of clonogenic common lymphoid progenitors in mouse bone marrow. Cell *91*, 661–672.

Krivtsov, A.V., and Armstrong, S.A. (2007). MLL translocations, histone modifications and leukaemia stem-cell development. Nat. Rev. Cancer *7*, 823–833.

Kyba, M., Perlingeiro, R.C., and Daley, G.Q. (2002). HoxB4 confers definitive lymphoid-myeloid engraftment potential on embryonic stem cell and yolk sac hematopoietic progenitors. Cell *109*, 29–37.

La Motte-Mohs, R.N., Herer, E., and Zuniga-Pflucker, J.C. (2004). Induction of T cell development from human cord blood hematopoietic stem cells by Delta-like 1 in vitro. Blood *105*, 1431–1439.

Lagresle-Peyrou, C., Six, E.M., Picard, C., Rieux-Laucat, F., Michel, V., Ditadi, A., Demerens-de Chappedelaine, C., Morillon, E., Valensi, F., Simon-Stoos, K.L., et al. (2009). Human adenylate kinase 2 deficiency causes a profound hematopoietic defect associated with sensorineural deafness. Nat. Genet. *41*, 106–111.

Lansdorp, P.M., Sutherland, H.J., and Eaves, C.J. (1990). Selective expression of CD45 isoforms on functional subpopulations of CD34+ hemopoietic cells from human bone marrow. J. Exp. Med. *172*, 363–366.

Lapidot, T., Pflumio, F., Doedens, M., Murdoch, B., Williams, D.E., and Dick, J.E. (1992). Cytokine stimulation of multilineage hematopoiesis from immature human cells engrafted in *scid* mice. Science *255*, 1137–1141.

Larochelle, A., Savona, M., Wiggins, M., Anderson, S., Ichwan, B., Keyvanfar, K., Morrison, S.J., and Dunbar, C.E. (2011). Human and rhesus macaque hematopoietic stem cells cannot be purified based only on SLAM family markers. Blood *117*, 1550–1554.

Lathia, J.D., Gallagher, J., Heddleston, J.M., Wang, J., Eyler, C.E., Macswords, J., Wu, Q., Vasanji, A., McLendon, R.E., Hjelmeland, A.B., et al. (2010). Integrin alpha 6 regulates glioblastoma stem cells. Cell Stem Cell *6*, 421–432.

Lessard, J., and Sauvageau, G. (2003). Bmi-1 determines the proliferative capacity of normal and leukaemic stem cells. Nature *423*, 255–260.

Lorenz, E., Uphoff, D., Reid, T.R., and Shelton, E. (1951). Modification of irradiation injury in mice and guinea pigs by bone marrow injections. J. Natl. Cancer Inst. *12*, 197–201.

Maillard, I., Koch, U., Dumortier, A., Shestova, O., Xu, L., Sai, H., Pross, S.E., Aster, J.C., Bhandoola, A., Radtke, F., et al. (2008). Canonical notch signaling is dispensable for the maintenance of adult hematopoietic stem cells. Cell Stem Cell *2*, 356–366.

Majeti, R., Park, C.Y., and Weissman, I.L. (2007). Identification of a hierarchy of multipotent hematopoietic progenitors in human cord blood. Cell Stem Cell *1*, 635–645.

Mansson, R., Hultquist, A., Luc, S., Yang, L., Anderson, K., Kharazi, S., Al-Hashmi, S., Liuba, K., Thoren, L., Adolfsson, J., et al. (2007). Molecular evidence for hierarchical transcriptional lineage priming in fetal and adult stem cells and multipotent progenitors. Immunity *26*, 407–419.

Manz, M.G., Miyamoto, T., Akashi, K., and Weissman, I.L. (2002). Prospective isolation of human clonogenic common myeloid progenitors. Proc. Natl. Acad. Sci. USA *99*, 11872–11877.

Maximow, A. (1909). Der Lymphozyt als gemeinsame Stammzelle der verschiedenen Blutelemente in der embryonalen Entwicklung und im postfetalen Leben der Säugetiere. Folia Haematol. (Frankf.) *8*, 125–134.

Mazurier, F., Doedens, M., Gan, O.I., and Dick, J.E. (2003). Rapid myeloerythroid repopulation after intrafemoral transplantation of NOD-SCID mice reveals a new class of human stem cells. Nat. Med. *9*, 959–963.

McCune, J.M., Namikawa, R., Kaneshima, H., Shultz, L.D., Lieberman, M., and Weissman, I.L. (1988). The SCID-hu mouse: murine model for the analysis of human hematolymphoid differentiation and function. Science *241*, 1632–1639.

Mestas, J., and Hughes, C.C. (2004). Of mice and not men: differences between mouse and human immunology. J. Immunol. *172*, 2731–2738.

Miller, C.L., Audet, J., and Eaves, C.J. (2002). Ex vivo expansion of human and murine hematopoietic stem cells. Methods Mol. Med. *63*, 189–208.

Milyavsky, M., Gan, O.I., Trottier, M., Komosa, M., Tabach, O., Notta, F., Lechman, E., Hermans, K.G., Eppert, K., Konovalova, Z., et al. (2010). A distinctive DNA damage response in human hematopoietic stem cells reveals an apoptosis-independent role for p53 in self-renewal. Cell Stem Cell *7*, 186–197.

Mohrin, M., Bourke, E., Alexander, D., Warr, M.R., Barry-Holson, K., Le Beau, M.M., Morrison, C.G., and Passegue, E. (2010). Hematopoietic stem cell quiescence promotes error-prone DNA repair and mutagenesis. Cell Stem Cell *7*, 174–185.

Moore, M.A., Williams, N., and Metcalf, D. (1973). In vitro colony formation by normal and leukemic human hematopoietic cells: characterization of the colony-forming cells. J. Natl. Cancer Inst. *50*, 603–623.

Morrison, S.J., Wandycz, A.M., Hemmati, H.D., Wright, D.E., and Weissman, I.L. (1997). Identification of a lineage of multipotent hematopoietic progenitors. Development *124*, 1929–1939.

Mosier, D.E., Gulizia, R.J., Baird, S.M., and Wilson, D.B. (1988). Transfer of a functional human immune system to mice with severe combined immunodeficiency. Nature *335*, 256–259.

Mosier, D., Gulizia, R., Baird, S., Wilson, D., Spector, D., and Spector, S. (1991). Human Immunodeficiency virus infection of human-PBL-SCID mice. Science *251*, 791–794.

Mullighan, C.G., Su, X., Zhang, J., Radtke, I., Phillips, L.A., Miller, C.B., Ma, J., Liu, W., Cheng, C., Schulman, B.A., et al. (2009). Deletion of IKZF1 and prognosis in acute lymphoblastic leukemia. N. Engl. J. Med. *360*, 470–480.

Murray, L., Chen, B., Galy, A., Chen, S., Tushinski, R., Uchida, N., Negrin, R., Tricot, G., Jagannath, S., Vesole, D., et al. (1995). Enrichment of human hematopoietic stem cell activity in the CD34+Thy-1+Lin- subpopulation from mobilized peripheral blood. Blood *85*, 368–378.

Namikawa, R., Kaneshima, H., Lieberman, M., Weissman, I.L., and McCune, J.M. (1988). Infection of the SCID-hu mouse by HIV-1. Science *242*, 1684–1686.

Negrin, R.S., Atkinson, K., Leemhuis, T., Hanania, E., Juttner, C., Tierney, K., Hu, W.W., Johnston, L.J., Shizurn, J.A., Stockerl-Goldstein, K.E., et al. (2000). Transplantation of highly purified CD34+Thy-1+ hematopoietic stem cells in patients with metastatic breast cancer. Biol. Blood Marrow Transplant. *6*, 262–271.

Neumuller, R.A., and Knoblich, J.A. (2009). Dividing cellular asymmetry: asymmetric cell division and its implications for stem cells and cancer. Genes Dev. *23*, 2675–2699.

Niebuhr, B., Iwanski, G.B., Schwieger, M., Roscher, S., Stocking, C., and Cammenga, J. (2009). Investigation of C/EBPalpha function in human (versus murine) myelopoiesis provides novel insight into the impact of CEBPA mutations in acute myelogenous leukemia (AML). Leukemia *23*, 978–983.

Noguchi, M., Yi, H., Rosenblatt, H.M., Filipovich, A.H., Adelstein, S., Modi, W.S., McBride, O.W., and Leonard, W.J. (1993). Interleukin-2 receptor gamma chain mutation results in X-linked severe combined immunodeficiency in humans. Cell *73*, 147–157.

North, T.E., Goessling, W., Walkley, C.R., Lengerke, C., Kopani, K.R., Lord, A.M., Weber, G.J., Bowman, T.V., Jang, I.H., Grosser, T., et al. (2007). Prostaglandin E2 regulates vertebrate haematopoietic stem cell homeostasis. Nature *447*, 1007–1011.

Notta, F., Doulatov, S., and Dick, J.E. (2010). Engraftment of human hematopoietic stem cells is more efficient in female NOD/SCID/IL-2Rgc-null recipients. Blood *115*, 3704–3707.

Notta, F., Doulatov, S., Laurenti, E., Poeppl, A., Jurisica, I., and Dick, J.E. (2011). Isolation of single human hematopoietic stem cells capable of long-term multilineage engraftment. Science *333*, 218–221.

Novershtern, N., Subramanian, A., Lawton, L.N., Mak, R.H., Haining, W.N., McConkey, M.E., Habib, N., Yosef, N., Chang, C.Y., Shay, T., et al. (2011). Densely interconnected transcriptional circuits control cell states in human hematopoiesis. Cell *144*, 296–309.

Ohishi, K., Varnum-Finney, B., and Bernstein, I.D. (2002). Delta-1 enhances marrow and thymus repopulating ability of human CD34(+)CD38(-) cord blood cells. J. Clin. Invest. *110*, 1165–1174.

Orkin, S.H., and Zon, L.I. (2008). Hematopoiesis: an evolving paradigm for stem cell biology. Cell *132*, 631–644.

Osawa, M., Hanada, K., Hamada, H., and Nakauchi, H. (1996). Long-term lymphohematopoietic reconstitution by a single CD34-low/negative hematopoietic stem cell. Science *273*, 242–245.

Owen, C., Barnett, M., and Fitzgibbon, J. (2008). Familial myelodysplasia and acute myeloid leukaemia–a review. Br. J. Haematol. *140*, 123–132.

Pajcini, K.V., Speck, N.A., and Pear, W.S. (2011). Notch signaling in mammalian hematopoietic stem cells. Leukemia *25*, 1525–1532.

Pike, B.L., and Robinson, W.A. (1970). Human bone marrow colony growth in agar-gel. J. Cell. Physiol. *76*, 77–84.

Prieyl, J.A., and LeBien, T.W. (1996). Interleukin 7 independent development of human B cells. Proc. Natl. Acad. Sci. USA *93*, 10348–10353.

Puel, A., Ziegler, S.F., Buckley, R.H., and Leonard, W.J. (1998). Defective IL7R expression in T(-)B(+)NK(+) severe combined immunodeficiency. Nat. Genet. *20*, 394–397.

Ramalho-Santos, M., Yoon, S., Matsuzaki, Y., Mulligan, R.C., and Melton, D.A. (2002). "Stemness": transcriptional profiling of embryonic and adult stem cells. Science *298*, 597–600.

Raya, A., Rodriguez-Piza, I., Guenechea, G., Vassena, R., Navarro, S., Barrero, M.J., Consiglio, A., Castella, M., Rio, P., Sleep, E., et al. (2009). Disease-corrected haematopoietic progenitors from Fanconi anaemia induced pluripotent stem cells. Nature *460*, 53–59.

Raymond, K., Deugnier, M.A., Faraldo, M.M., and Glukhova, M.A. (2009). Adhesion within the stem cell niches. Curr. Opin. Cell Biol. *21*, 623–629.

Reya, T., Morrison, S.J., Clarke, M.F., and Weissman, I.L. (2001). Stem cells, cancer, and cancer stem cells. Nature *414*, 105–111.

Reya, T., Duncan, A.W., Ailles, L., Domen, J., Scherer, D.C., Willert, K., Hintz, L., Nusse, R., and Weissman, I.L. (2003). A role for Wnt signalling in self-renewal of haematopoietic stem cells. Nature *423*, 409–414.

Richie Ehrlich, L.I., Serwold, T., and Weissman, I.L. (2011). In vitro assays misrepresent in vivo lineage potentials of murine lymphoid progenitors. Blood *117*, 2618–2624.

Rizo, A., Dontje, B., Vellenga, E., de Haan, G., and Schuringa, J.J. (2008). Long-term maintenance of human hematopoietic stem/progenitor cells by expression of BMI1. Blood *111*, 2621–2630.

Rizo, A., Olthof, S., Han, L., Vellenga, E., de Haan, G., and Schuringa, J.J. (2009). Repression of BMI1 in normal and leukemic human CD34(+) cells impairs self-renewal and induces apoptosis. Blood *114*, 1498–1505.

Rodrigues, N.P., Janzen, V., Forkert, R., Dombkowski, D.M., Boyd, A.S., Orkin, S.H., Enver, T., Vyas, P., and Scadden, D.T. (2005). Haploinsufficiency of GATA-2 perturbs adult hematopoietic stem-cell homeostasis. Blood *106*, 477–484.

Rongvaux, A., Willinger, T., Takizawa, H., Rathinam, C., Auerbach, W., Murphy, A.J., Valenzuela, D.M., Yancopoulos, G.D., Eynon, E.E., Stevens, S., et al. (2011). Human thrombopoietin knockin mice efficiently support human hematopoiesis in vivo. Proc. Natl. Acad. Sci. USA *108*, 2378–2383.

Rossi, M.I., Yokota, T., Medina, K.L., Garrett, K.P., Comp, P.C., Schipul, A.H., Jr., and Kincade, P.W. (2003). B lymphopoiesis is active throughout human life, but there are developmental age-related changes. Blood *101*, 576–584.

Rossi, D.J., Jamieson, C.H., and Weissman, I.L. (2008). Stems cells and the pathways to aging and cancer. Cell *132*, 681–696.

Sakai, R., Kajiume, T., Inoue, H., Kanno, R., Miyazaki, M., Ninomiya, Y., and Kanno, M. (2003). TCDD treatment eliminates the long-term reconstitution activity of hematopoietic stem cells. Toxicol. Sci. *72*, 84–91.

Sakamoto, K., Yamaguchi, S., Ando, R., Miyawaki, A., Kabasawa, Y., Takagi, M., Li, C.L., Perbal, B., and Katsube, K. (2002). The nephroblastoma overexpressed gene (NOV/ccn3) protein associates with Notch1 extracellular domain and inhibits myoblast differentiation via Notch signaling pathway. J. Biol. Chem. *277*, 29399–29405.

Sankaran, V.G., Xu, J., Ragoczy, T., Ippolito, G.C., Walkley, C.R., Maika, S.D., Fujiwara, Y., Ito, M., Groudine, M., Bender, M.A., et al. (2009). Developmental and species-divergent globin switching are driven by BCL11A. Nature *460*, 1093–1097.

Saran, N., Lyszkiewicz, M., Pommerencke, J., Witzlau, K., Vakilzadeh, R., Ballmaier, M., von Boehmer, H., and Krueger, A. (2010). Multiple extrathymic precursors contribute to T-cell development with different kinetics. Blood *115*, 1137–1144.

Schlenner, S.M., Madan, V., Busch, K., Tietz, A., Laufle, C., Costa, C., Blum, C., Fehling, H.J., and Rodewald, H.R. (2010). Fate mapping reveals separate origins of T cells and myeloid lineages in the thymus. Immunity *32*, 426–436.

Schuringa, J.J., and Vellenga, E. (2010). Role of the polycomb group gene BMI1 in normal and leukemic hematopoietic stem and progenitor cells. Curr. Opin. Hematol. *17*, 294–299.

Schwieger, M., Lohler, J., Fischer, M., Herwig, U., Tenen, D.G., and Stocking, C. (2004). A dominant-negative mutant of C/EBPalpha, associated with acute myeloid leukemias, inhibits differentiation of myeloid and erythroid progenitors of man but not mouse. Blood *103*, 2744–2752.

Shepherd, B.E., Guttorp, P., Lansdorp, P.M., and Abkowitz, J.L. (2004). Estimating human hematopoietic stem cell kinetics using granulocyte telomere lengths. Exp. Hematol. *32*, 1040–1050.

Shojaei, F., Trowbridge, J., Gallacher, L., Yuefei, L., Goodale, D., Karanu, F., Levac, K., and Bhatia, M. (2005). Hierarchical and ontogenic positions serve to define the molecular basis of human hematopoietic stem cell behavior. Dev. Cell *8*, 651–663.

Shortman, K., and Naik, S.H. (2007). Steady-state and inflammatory dendritic-cell development. Nat. Rev. Immunol. *7*, 19–30.

Shultz, L., Schweitzer, P., Christianson, S., Gott, B., Schweitzer, I., Tennent, B., McKenna, S., Mobraaten, L., Rajan, T., Greiner, D., et al. (1995). Multiple defects in innate and adaptive immunological function in NOD/LtSz-*scid* mice. J. Immunol. *154*, 180–191.

Shultz, L.D., Lyons, B.L., Burzenski, L.M., Gott, B., Chen, X., Chaleff, S., Kotb, M., Gillies, S.D., King, M., Mangada, J., et al. (2005). Human lymphoid and myeloid cell development in NOD/LtSz-scid IL2R gamma null mice engrafted with mobilized human hemopoietic stem cells. J. Immunol. *174*, 6477–6489.

Shultz, L.D., Ishikawa, F., and Greiner, D.L. (2007). Humanized mice in translational biomedical research. Nat. Rev. Immunol. *7*, 118–130.

Sitnicka, E., Buza-Vidas, N., Larsson, S., Nygren, J.M., Liuba, K., and Jacobsen, S.E. (2003). Human CD34+ hematopoietic stem cells capable of multilineage engrafting NOD/SCID mice express flt3: distinct flt3 and c-kit expression and response patterns on mouse and candidate human hematopoietic stem cells. Blood *102*, 881–886.

Six, E.M., Bonhomme, D., Monteiro, M., Beldjord, K., Jurkowska, M., Cordier-Garcia, C., Garrigue, A., Dal Cortivo, L., Rocha, B., Fischer, A., et al. (2007). A human postnatal lymphoid progenitor capable of circulating and seeding the thymus. J. Exp. Med. *204*, 3085–3093.

Spangrude, G.J., Heimfeld, S., and Weissman, I.L. (1988). Purification and characterization of mouse hematopoietic stem cells. Science *241*, 58–62.

Stingl, J., Eaves, C.J., Kuusk, U., and Emerman, J.T. (1998). Phenotypic and functional characterization in vitro of a multipotent epithelial cell present in the normal adult human breast. Differentiation *63*, 201–213.

Sutherland, H., Eaves, C., Eaves, A., Dragowska, W., and Lansdorp, P. (1989). Characterization and partial purification of human marrow cells capable of initiating long-term hematopoiesis in vitro. Blood *74*, 1563–1570.

Sutherland, H.J., Eaves, C.J., Lansdorp, P.M., Thacker, J.D., and Hogge, D.E. (1991). Differential regulation of primitive human hematopoietic cells in long-term cultures maintained on genetically engineered murine stromal cells. Blood *78*, 666–672.

Takenaka, K., Prasolava, T.K., Wang, J.C., Mortin-Toth, S.M., Khalouei, S., Gan, O.I., Dick, J.E., and Danska, J.S. (2007). Polymorphism in Sirpa modulates engraftment of human hematopoietic stem cells. Nat. Immunol. *8*, 1313–1323.

Tenen, D.G. (2003). Disruption of differentiation in human cancer: AML shows the way. Nat. Rev. Cancer *3*, 89–101.

Till, J.E., and McCulloch, E.A. (1961). A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. Radiat. Res. *14*, 213–222.

Till, J.E., McCulloch, E.A., and Siminovitch, L. (1964). A Stochastic Model of Stem Cell Proliferation Based on the Growth of Spleen Colony-Forming Cells. Proc. Natl. Acad. Sci. USA *51*, 29–36.

Trowbridge, J.J., Xenocostas, A., Moon, R.T., and Bhatia, M. (2006). Glycogen synthase kinase-3 is an in vivo regulator of hematopoietic stem cell repopulation. Nat. Med. *12*, 89–98.

Tsai, F.Y., and Orkin, S.H. (1997). Transcription factor GATA-2 is required for proliferation/survival of early hematopoietic cells and mast cell formation, but not for erythroid and myeloid terminal differentiation. Blood *89*, 3636–3643.

van der Meer, L.T., Jansen, J.H., and van der Reijden, B.A. (2010). Gfi1 and Gfi1b: key regulators of hematopoiesis. Leukemia *24*, 1834–1843.

van Furth, R., and Cohn, Z.A. (1968). The origin and kinetics of mononuclear phagocytes. J. Exp. Med. *128*, 415–435.

Varnum-Finney, B., Xu, L., Brashem-Stein, C., Nourigat, C., Flowers, D., Bakkour, S., Pear, W.S., and Bernstein, I.D. (2000). Pluripotent, cytokine-dependent, hematopoietic stem cells are immortalized by constitutive Notch1 signaling. Nat. Med. *6*, 1278–1281.

Vinh, D.C., Patel, S.Y., Uzel, G., Anderson, V.L., Freeman, A.F., Olivier, K.N., Spalding, C., Hughes, S., Pittaluga, S., Raffeld, M., et al. (2010). Autosomal dominant and sporadic monocytopenia with susceptibility to mycobacteria, fungi, papillomaviruses, and myelodysplasia. Blood *115*, 1519–1529.

Vogel, W., Scheding, S., Kanz, L., and Brugger, W. (2000). Clinical applications of CD34(+) peripheral blood progenitor cells (PBPC). Stem Cells *18*, 87–92.

Wada, H., Masuda, K., Satoh, R., Kakugawa, K., Ikawa, T., Katsura, Y., and Kawamoto, H. (2008). Adult T-cell progenitors retain myeloid potential. Nature *452*, 768–772.

Wang, J.C., Doedens, M., and Dick, J.E. (1997). Primitive human hematopoietic cells are enriched in cord blood compared with adult bone marrow or mobilized peripheral blood as measured by the quantitative in vivo SCID-repopulating cell assay. Blood *89*, 3919–3924.

Wang, L., Menendez, P., Shojaei, F., Li, L., Mazurier, F., Dick, J.E., Cerdan, C., Levac, K., and Bhatia, M. (2005). Generation of hematopoietic repopulating cells from human embryonic stem cells independent of ectopic HOXB4 expression. J. Exp. Med. *201*, 1603–1614.

Wei, J., Wunderlich, M., Fox, C., Alvarez, S., Cigudosa, J.C., Wilhelm, J.S., Zheng, Y., Cancelas, J.A., Gu, Y., Jansen, M., et al. (2008). Microenvironment determines lineage fate in a human model of MLL-AF9 leukemia. Cancer Cell *13*, 483–495.

Welner, R.S., Pelayo, R., and Kincade, P.W. (2008). Evolving views on the genealogy of B cells. Nat. Rev. Immunol. *8*, 95–106.

Weng, A.P., Ferrando, A.A., Lee, W., Morris, J.P.t., Silverman, L.B., Sanchez-Irizarry, C., Blacklow, S.C., Look, A.T., and Aster, J.C. (2004). Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. Science *306*, 269–271.

Willinger, T., Rongvaux, A., Strowig, T., Manz, M.G., and Flavell, R.A. (2011). Improving human hemato-lymphoid-system mice by cytokine knock-in gene replacement. Trends Immunol. *32*, 321–327.

Wilson, A., Laurenti, E., Oser, G., van der Wath, R.C., Blanco-Bose, W., Jaworski, M., Offner, S., Dunant, C.F., Eshkind, L., Bockamp, E., et al. (2008). Hematopoietic stem cells reversibly switch from dormancy to self-renewal during homeostasis and repair. Cell *135*, 1118–1129.

Zhang, C.C., Kaba, M., Iizuka, S., Huynh, H., and Lodish, H.F. (2008). Angiopoietin-like 5 and IGFBP2 stimulate ex vivo expansion of human cord blood hematopoietic stem cells as assayed by NOD/SCID transplantation. Blood *111*, 3415–3423.