Published in final edited form as:
Semin Hematol. 2013 April ; 50(2): . doi:10.1053/j.seminhematol.2013.03.029.

Modeling human hematopoietic stem cell biology in the mouse

Stephen M. Sykes[1] and **David T. Scadden**[2]

[1]Research Institute of Fox Chase Cancer Center, Philadelphia, PA

[2]Center for Regenerative Medicine, Massachusetts General Hospital, Harvard Stem Cell Institute, Harvard University Department of Stem Cell and Regenerative Biology, Cambridge MA

Abstract

Hematopoietic stem cells (HSCs) have the immense task of supplying an organism with enough blood to sustain a lifespan. Much of what is known about how this scant population of cells can meet the varying demand of producing more than 10^{11} cells per day comes from studies conducted in an animal that is a fraction of our size and lives roughly $1/30^{th}$ of our lifespan. The differences in longevity can be expected to impose different demands on a cell essential for existence. It is therefore unsurprising that while the mouse has proven invaluable in defining the organizing principals of how hematopoiesis is governed, mediators of cell localization as well as a range of experimental methods, the differences in cell cycling, DNA repair and specific molecular features of hematopoietic stem cells in humans are evident and important. Here, the utility and drawbacks of the mouse as an experimental model for human hematopoietic stem cell biology are discussed.

Introduction

Hematopoietic stem cells (HSCs) represent the first identified and most well characterized adult stem cell. The clinical use of HSCs to treat a variety of human disorders and diseases has also made them a key building block in the foundation of regenerative medicine. Much of our understanding regarding the regulation and function of HSCs comes from decades of seminal studies performed in mice. Our goal is to briefly summarize the knowledge of HSC biology that has been productively gained from mouse models and to also note differences in HSC biology between mice and humans that may color the application of lessons from the mouse to human settings.

Developmental origin of HSCs

The hematopoietic stem cell (HSC) is defined as a multipotent cell capable of both duplicating itself (self-renew) as well as generating all the mature cell lineages that comprise the blood. The pool of adult HSCs required to support the hematopoietic system over the lifespan of both mice and humans are generated by the mesoderm during embryogenesis. Several distinct pools of hematopoietic stem and progenitors (HSPCs) have been identified at different stages and anatomical locations throughout embryogenesis and while most extensively characterized in the mouse, it appears parallel processes occur in humans

Correspondence: Stephen Sykes, PhD, Research Institute of Fox Chase Cancer Center, Immune Cell Development and Host Defense, 333 Cottman Avenue, Philadelphia, PA; Stephen_Sykes@fccc.edu. David Scadden MD, 185 Cambridge St, Boston, MA 02114; David_Scadden@harvard.edu.

The prevailing consensus about the cellular origin of primitive HSCs in vertebrates is that they are produced by a mesodermal precursor cell, called the hemangioblast, which gives rise to both hematopoietic and endothelial lineages. [1,2] Anatomically, the earliest sign of hematopoietic activity in both humans and mice can be found in the yolk sac. These hematopoietic progenitors are lineage restricted, capable of selectively producing myeloid and erythroid cells and lack the ability to support long-term hematopoiesis. [34] In the embryo proper, definitive HSCs first emerge in the aorta-gonad-mesonephros (AGM) region of both humans and mice as well as the vitelline and umbilical arteries of the murine embryo.[5,6] Additionally, primitive HSCs have also been found in the placentas of humans and mice indicating that extra-embryonic tissue may also be an important contributor of the HSC pool. Whether these HSC pools develop independently or are produced in a common location and subsequently migrate to distinct residences remains controversial. The definitive HSCs of the AGM, and possibly the extra-embryonic tissue, colonize the fetal liver where they undergo extensive cell division expanding the HSC pool. These cells migrate through the blood to the nascent bone marrow when ossification is first occurring and provide lifelong hematopoiesis. Their trafficking from bone marrow to blood and back appears to continue throughout life and the canonical processes of trafficking appear to be shared at both the cellular and molecular level; an issue of clinical importance discussed below.

The existence of the HSC

Perhaps the most compelling evidence of the utility of the mouse model for human biology is in the experimental definition of the hematopoietic stem cell (HSC). The initial proposition that a stem cell provided the basis for all blood cells was the ‘unifying hypothesis for hematopoiesis’ of Artur Pappenheim in 1905.[7] The concept was an intellectual construct of relatively little consequence until the advent of nuclear weapons in WWII. In that context, the possibility of stem cells as a countermeasure against the devastating consequences of radiation on blood cell production greatly increased interest in determining whether the entity actually existed.

At that time, there was still debate about the existence of a stem cell with the competing hypothesis proposing that cells were capable of regenerating themselves, a notion largely driven by observations about tumor cells. Canadian scientists James Till and Ernest McCulloch who were determining the number of untreated donor bone marrow cells required to rescue mice exposed to supralethal doses of radiation capitalized on the observation that transplanted recipients develop gross splenic nodules.[8,9] In collaboration with Drs. Andrew Becker and Louis Siminovitch, Till and McCulloch established that a select progenitor population of hematopoietic cells can self-renewal and expand clonally to give rise to mature lineages.[9,10] They hypothesized that the splenic nodules were the result of an individual cell engrafting in the spleen and expanding to form a colony, much in the same way colonies of organisms grew in microbiologic assays. To formally prove the clonal origin of the nodules, Till and McCulloch performed a brilliant experiment in which they transplanted cells from an animal into a lethally irradiated recipient, exposed the recipient to sub-lethal doses of radiation sufficient to induce karyotypic alterations thereby creating a genetic mark in cells. By painstakingly examining the chromosomal spreads on individual nodules, they showed that within a given nodule, the karyotypic abnormality was consistently present, but that it was different in a neighboring nodule. Further, the nodules contained mature blood cells and, in a separate set of experiments, were shown to be capable of transplantation into a second irradiated host. The cardinal features of stem cells, differentiation and self-renewal were therefore experimentally defined.

These studies were a landmark for the study of hematopoiesis and they provided the scientific basis for continuing clinical bone marrow transplant efforts in people even after the early days of that area of medicine were cast in deep shadow by high rates of morbidity and mortality. Importantly, these studies also provided the gold standard for experimentally defining stem cells in other tissues and firmly established the mouse as a model in which such experiments could be effectively conducted.

Determining the face of the HSC

Though the existence of the HSC was realized in the 1960s, their rarity and indistinguishable morphology stymied their identification for nearly 25 years. The advent of monoclonal antibody production and laser based instruments to quantify and isolate cells based on antibody based fluorescent tags (fluorescence activated cell sorting or FACS) were powerfully put to use by Dr. Irving Weissman and others to determine if stem cells had distinctive surface antigens. Leveraging the transplant model, they took subsets of cells isolated by virtue of distinct antibody binding profiles and defined those where blood repopulating ability was enriched. This strategy provided the first effective means of segregating repopulating from non-repopulating fractions from a heterogeneous mixture of bone marrow mononuclear cells.[11] This approach has been repeated by a number of laboratories in a manner resembling the peeling of an onion to gain progressively more enriched stem cell populations. These populations have been marked by expression of cKit/CD117[12], CD150[13] and the absence of CD34, Flk-2/Flt3, CD48 and CD244.[14,15] We also note that murine HSCs have also been segregated based on their ability to efflux dyes (Rhodamine) as well as their cell cycle state.[16–18]

Using surface-protein profiles to purify mouse HSCs laid the foundation for identifying the cognate human HSC profile, but the process was encumbered by the absence of a solid assay for human HSC. This technical limitation was partially overcome by the pioneering work of John Dick who established xenotransplant models where multi-immunodeficient mice could be reliably engrafted by human hematopoietic cells.[19] This advance enabled the testing of *in vivo* repopulating ability of subsets of human hematopoietic cells, albeit without the efficiencies associated with mouse-mouse transplant assays. Comparison of the surface marker profiles of cell subsets enriched for repopulating ability indicated that antigens like c-kit and the absence of lineage specific antigens were shared between human and mouse repopulating cells. However, it made apparent how distinctive the human antigen signature is. For example, while CD34 expression is present on human repopulating cells, its expression is minimal on mouse repopulating cells and marks more differentiated progenitors.[14] Also, there is no good paralogue for some markers; Sca-1 is very useful in mouse HSC isolation, but no such molecule exists in human. The ‘SLAM’ signature of antigens so useful for mouse HSC is not sufficient for isolating human or non-human primate HSC.[20] Therefore, the mouse has provided the method to identify cell surface signature molecules on human HSC, but the specific signatures are divergent.

There are indications that biologic differences that are not simply due to divergent ‘marker’ gene expression. This is evident with cell isolation strategies based on function. For example, the exclusion or extrusion of dyes such as rhodamine or Hoescht33342 was noted to be a property of the mouse HSC and attributed to differential ABC transporter activity.[18,21] This led to a highly useful means of isolating HSCs in mouse based on a so-termed “side population” when FACS sorting Hoescht33342 stained bone marrow cells. However, similar gating on primate cells is not as enriching. Conversely, isolating HSCs based on resistance to cytokine and anti-metabolite exposure ex vivo, can be used on slowly turning over human bone marrow cells, but not the more rapidly cycling mouse cells.[22]

These differences reflect distinct functional attributes that encourage caution when extending results in the mouse to the human.

HSC number

The ability to identify stem cells by their reconstituting function and their flow cytometric features has led to efforts to quantify stem cells in bone marrow and in the whole organism. In a series of elegant studies, Janis Abkowitz and colleagues used quantification by transplant to define the total body number of HSC in the mouse and the cat.[23] Notably, a comparable number of HSC were calculated. Given the difference in the total number of blood cells between the mouse and much larger, much longer lived mammal, the authors concluded that there must be marked differences in the number of descendent cells that could be generated per HSC in the cat and in the relative turnover time of the HSC pool. These studies further prompted attention to whether HSC cell cycle kinetics in primates were very different than those calculated for the mouse.

HSC cycling

The ability of humans to tolerate repeated cycles of continuously infused cell cycle specific cytotoxic agents such as 5-fluorouracil led to reasonable conjecture that HSC are largely quiescent, non-cycling cells. However, the mouse model suggested otherwise. While the quiescent fraction of the bone marrow was enriched in HSC and immunophenotypically isolated HSC expressed cycle restricting genes relative to progenitor cells, other data indicated that this relative difference was not reflective of deep quiescence.[24,25] The thymidine analog, BrdU, that is selectively incorporated by cells during the DNA synthesis phase (S-phase) of the cell cycle was used to demonstrate that mouse HSC cycle asynchronously with approximately 8% of HSC cycling at any given time and that HSCs divide between 30 and 57 days.[26,27] Additional studies using a refined definition of murine HSCs have suggested that BrdU-labeling is sub-optimal due to toxicity [28] and studies using inducible Histone H2B-GFP fusion protein to label cell divisions determined that there is heterogeneity within the stem cell pool with a subset of HSC turning over rapidly but a more dormant portion dividing only every 145 days.[29,30]

HSC cycling in non-human primates was examined using BrdU uptake and indicated that indeed cycling times were markedly longer than those found when similar methods were applied to the mouse with the frequency of HSC division estimated to be only once per year in baboons.[26,27,31] The principles of hemizygous selection in which the drift in the distribution of X-chromsome inactivation, is assessed over time and used to estimate the activity of HSC was applied to a cohort of over 1200 women by the Abkowitz laboratory to determine the replication rate of human HSCs.[32] Using several mathematical models, human HSCs are estimated to replicate once every 40 weeks on average.[32] These data starkly contrast with replication rates of murine HSCs (once every 2.5 weeks) or cats (once per 8.3 weeks) when assessed by similar methods. The data do indicate that the number of cell division expected over the lifetime of the respective animals would therefore be approximately equivalent. However, they imply that the relative contribution of active HSCs to quiescent clones is dramatically different among animals. The steady state ratio of active clones contributing to hematopoiesis compared with quiescent clones is approximately 1.46 for mice, 0.677 for cats and 0. 116 for humans. Therefore, human cycling kinetics and population dynamics are not well recapitulated in the mouse. The mouse has been a superb model for enabling questions about cycling and population dynamics to be raised and experimentally tested, but extension of the results from the mouse to the human is done with peril. Cycling and clonal activity are distinctly different between animals. It therefore cannot be assumed that the molecular phenomena regulating these events is identical. The place

where HSC regulation is conducted is the bone marrow in a specialized microenvironment termed the niche.

The HSC niche

When Till and McCulloch were experimentally defining the HSC other laboratories were examining where they resided and how they might be grown. Experiments using lead shielding specific anatomic sites demonstrated that blood repopulating cells did indeed reside in bones[33] and removal of central marrow of long bones indicated that primitive hematopoietic cells resided in close proximity to the endosteal bone surface.[34] Dexter used *ex vivo* culture systems to show that primitive hematopoietic cells depended on the non-hematopoietic cells of 'stroma' for their persistence.[35] But it was Raymond Schofield who first posed the notion that the HSC depended upon a specialized niche based on his observations using the Till and McCulloch method of stem cell enumeration. [36] He found that the putative stem cells generating colonies in the spleen were less robust at repopulating irradiated animals if derived from the spleen than if derived from the bone marrow. He therefore articulated the niche hypothesis that there is an anatomically definable microenvironment that governs the persistence and regulated differentiation of stem cells. He had no experimental system by which to test the hypothesis, but using observational evidence derived from the mouse, provided a central principal for stem cell biology that appears to be valid in far more than murine restricted systems.

Reasoning that bone regulates bone marrow, simple modifications of specific bone cells were tested by our laboratory and independently (using a more generalized model since shown to be consistent with modification of a bone stem/progenitor cell) the laboratory of Linheng Li and both documented that altered osteolineage cell activity resulted in altered primitive hematopoietic cell number.[37,38] These data provided support for the niche hypothesis in mammals and prompted extensive assessment for cells contributing to HSC regulation in the bone marrow of mice.

A number of elegant murine-based studies, have since shown that many different cell types contribute to the bone marrow HSC niche. These range from endothelial cells to multiple mesenchymal cells, to neural cells to blood cells.[39–46] Among mesenchymal cells there are those of osteolineage, more immature Nestin-postive-mesenchymal stem cells (MSCs), Leptin receptor positive perivascular cells and interstitial stellate cells positively affecting HSC while adipocytes have been shown to negatively influence HSC number.[47–50] In addition, studies have indicated that more mature osteoblasts participate in the ability of G-CSF to mobilize HSC.[51] Whether these populations participate in regulating human HSC number or function is still to be defined.

Efforts are underway to identify correspondent cell types between mouse and human bone marrow stroma. For example, the Nestin expressing mesenchymal cell found in mouse, is found in neonatal human bone marrow and there is evidence that it may be capable of regulating hematopoietic cells. (Frenette: personal communication; in press) The ability to define human homologues of the cells regulating hematopoiesis in the mouse may be of particular importance in three contexts defined by studies in mouse models. The first is localization, a process important in being able to both mobilize stem cells for harvesting and in the ability to engraft stem cells, both highly relevant for clinical hematopoietic stem cell transplantation. The second, in the ability to augment niche effects on HSC number as a means of altering regeneration and the third, as a participant in hematopoietic disease. Each of these has been documented in the mouse and each has been shown to be altered by drug-based interactions. Therefore the rationale provided by the mouse is strong impetus for human investigation.

Stem cell localization

Stem cells capable of repopulating an animal were first described to be in the blood of adult animals in the mouse. The ability to use blood as a source of stem cells for transplantation was constrained by the number of cells in the blood under steady-state conditions and therefore, it was of great interest when specific cytokines were found to augment HSC numbers in the blood. These studies resulted in the testing and ultimate adoption of G-CSF mobilization as common clinical practice to harvest stem cells for both autologous and allogeneic transplantation. The mouse provided the basis for beginning to unravel how G-CSF exerts this affect, providing tractable models for examining the histology of the marrow, manipulate specific subsets of cells and alter activity of pathways hypothesized to be relevant.

One such pathway is that of CXCR4/CXCL12 signaling. The CXCL12 (or SDF-1) chemokine was shown to be important for primitive hematopoietic cells through mouse studies in which animals engineered to be deficient in either the ligand or receptor had evidence of compromised hematopoiesis.[52,53] The CXCR4 deleted animal was particularly interesting because it did have evidence of immature hematopoietic cells in the blood, although transplantion of these progenitors could successfullyly engraft and repopulate of an irradiated recipient mouse. The first evidence for pharmacologic modulation of the CXCL12/CXCR4 axis as a means of mobilizing stem cells was from our laboratory where a variant of CXCL12 that caused persistent internalization of the CXCR4 receptor resulted in a 30-fold increase in primitive hematopoietic cells in the blood of mice.[54] Concurrent with these studies, pharmacologic modifiers of CXCR4 were being developed as possible anti-HIV medications given the dependence of a subset of HIV-1 on CXCR4 for lymphocyte infection. With additional mouse data indicating the potential for enhancing mobilization by inhibiting CXCR4, the impetus for testing CXCR4 antagonists in the clinic increased and resulted in eventual demonstration that such an approach could be clinically useful for individuals who had failed mobilization with G-CSF alone. The result of the mouse studies was therefore a useful pharmacologic agent in the hands of clinicians. This has also been now validated by targeting the α4β1 integrin, first observed to be important for HSC localization in the mouse.[55]

Localization phenomena could be understood and manipulated for increased harvesting of HSC, leading to the converse effect being studied namely enhancing stem cell delivery to the niche as a means of improving transplant efficiency. This issue was explored by an interesting combination of animal models. Leonard Zon's laboratory tested chemical libraries for their ability to increase hematopoietic stem cells in the zebrafish embryo. A modified version of prostaglandin E2 (dmPGE2) was found to increase fish hematopoiesis.[56] This was validated in mouse studies where HSC engraftment was enhanced 2-3-fold when the transplanted cells were exposed to dmPGE2 prior to infusion. A complementary mouse study in which deletion of the downstream signaling effector of PGE2 stimulation, Gαs, was deleted and shown to result in an absence of HSC in bone marrow.[57] These mouse studies propelled interest in using PGE2 in human transplantation. The clinical trials testing that approach are now underway with encouraging preliminary results. (Cutler: personal communication; under review) There are encouraging indications therefore that localization modeled in the mouse may be predictive of human biology and active testing of molecules defined in the mouse are likely to quickly transition to human studies.

Hematopoietic regeneration

Cytokine therapy for enhancing lineage specific cell types like red cells or granulocytes with recombinant erythropoietin or G-SCF respectively is so effective in the clinic that a similar approach has long been sought for HSC. However, despite decades of trying, no clinically useful mode of HSC expansion in vitro or in vivo has resulted. Therefore, modulating the niche rather than the HSC themselves has become an approach that has received some attention. It is in this setting that the finding of specific regulatory cells in the niche can be informative. For example, if osteolineage cells do participate in the niche, use of anabolic bone agents like recombinant parathyroid hormone may secondarily influence primitive hematopoietic cell function. This has been shown in the mouse including the ability to protect HSC from sequential cancer chemotherapy drug exposure and improvement in engraftment of limiting numbers of HSC.[58] Whether this can have an impact on patients is being tested.

Niche participation in disease

Some studies in the mouse have indicated that perturbing stroma cells can result in altered hematopoiesis. For example, altered Gαs signaling in osteocytes caused myeloid hyperproliferation,[59] *RARg* deletion in the microenvironment resulted in a myeloproliferative syndrome[60] and *CREB-binding protein* haploinsufficiency in the microenvironment also induced myeloproliferation with depletion of primitive hematopoietic cells.[61] Further, deletion of *Dicer1* in a specific subset of osteolineage cells in the bone marrow resulted in myelodysplasia and the rare emergence of acute myeloid leukemia.[62] The leukemia had intact *Dicer1*, but had acquired new, complex genotypic changes suggesting that the environment imposes selection criteria on the hematopoietic system and when the environment is perturbed, a highly pathologic hematopoietic system can result.

The potential for niche cells to impose fitness constraints on hematopoietic stem cells has also been demonstrated in the mouse with age. The laboratory of Hartmut Geiger has demonstrated that in aged mice, primitive hematopoietic cells have distinct physical relationships to architectural features of the bone marrow.[63] Cells were in closer proximity to the endosteum and had decreased adhesive characteristics when aged. Further, the same laboratory demonstrated that when equivalent numbers of HSC clones were transplanted into older mice compared with younger mice, the clonal diversity of the resulting hematopoiesis was much more constrained.[64] These data suggest that clonal selection is distinctive in the older niche and that a predisposition to a more monoclonal state may exist. These issues are ones of considerable interest in understanding how the niche may participate in disease pathogenesis, particularly dysplastic or neoplastic disease.

Determining whether similar phenomena occur in the human is clearly more difficult, but the mouse has inspired attention to the possibility in humans. The result is studies where altered TNFa and IL-32 production by stromal cells has been shown from patients with myelodysplastic syndrome (MDS)[65] and frequent cytogenetic abnormalities in marrow mesenchymal cells from patients with AML and MDS documented.[66] These studies are still early, but the mouse has provided a rationale for exploring them in detail, particularly when correspondent cells implicated in the mouse can be identified, isolated and examined from human samples.

HSC aging at a cellular level

Stem cells have been implicated in age related disease and poor regenerative function in most tissues. In blood, the frequency of MDS and leukemia clearly increases as does a

general decline in adaptive immune function. The latter issue has been particularly amenable to investigation in the mouse. Murine HSC change with age in specific ways. The immunophenotypic HSC population increases in at least some strains of mice, but there is disparity between immunophenotype and function. The number of functional repopulating cells increases only modestly so interpretation of results based on immunophenotype alone should be interpreted with caution. Since human HSC are defined by immunophenotype or the imperfect model of xenotransplant into an immunocompromised mouse, human HSC increases with age that have been reported should be considered with the caveat above.[67,68] Also changing in mice is the ability of aged HSCs to engraft upon transplant and this does seem to be consistent in human HSCs from older donors in at least some studies and in clinical experience.[69,70] The output of cells from a given HSC also seems to be decreased in the human, a finding quite different from what has been reported in mouse where production was not affected.[71] This has been hypothesized to be due to accumulated genetic injury over the longer lifespan of the human and does point to how differential longevity of the animal is reflected in issues like cell cycling (as above) and HSC function.

The issue of immune compromise with age is thought to be partially accounted for by poor lymphopoiesis relative to myelopoiesis in the elderly. This has again been studied in detail in the mouse and found to be related to shifts in the relative abundance of myeloid-biased versus lymphoid-biased HSC within the heterogeneous HSC pool. As mice age, the balanced lineage-bias diversity of the HSC pool seen in young mice skews toward an enrichment of myeloid-biased HSCs in accompaniment with a gene expression signature consistent with myeloid over lymphoid differentiation .[72,73] Human cells are not possible to study with the same rigor, but immunodeficient mice transplanted with Lin^{-}, $CD34^{+}$, $CD38^{-}$, $CD90^{+}$, $CD45RA^{-}$ human HSCs taken from young adults reconstitute both myeloid and lymphoid lineages, whereas the equivalent population derived from elderly donors can only restore myelopoiesis.66 However, it should be noted that similar experimental strategies using $CD34^{+}CD38^{-}$ purified human hematopoietic cells did not display an age-related lineage bias within this population. This disparity may potentially be resolved by performing similar assays using newly identified human HSCs markers, such as CD49f.[74]

HSC aging at a molecular level

The diminished function of aging cells is associated with several molecular changes such as telomere erosion, genomic instability and the accumulation intracellular free radicals. Gene expression analyses of both young and old HSCs from either species reveal that genes associated with inflammation and oxidative stress increase with age whereas genes contributing to DNA repair, chromatin structure and epigenetic regulation decline.[75] DNA damage is anticipated to accumulate with age in either species, but clear evidence in the mouse depended upon genetic inhibition of DNA repair pathways.[76] In contrast, studies of human cells show increased H2A.X foci and elevated activity of DNA-damage response (DDR) pathways (e.g. ATM, 53BP1 and MDC1).[77] Similar differences between species exist in DNA damage response pathways. In murine HSC, it has been shown that non-homologous end joining is the dominant mechanism of DNA repair, but this error-prone mechanism would be particularly undesirable in a long-lived organism like the human. Indeed, John Dick and colleagues found that in human HSC double strand break repair of DNA is delayed and p53 plays a critical role in mediating the repair process.

The largest molecular discrepancy between human and mice is telomere length. With every cell division telomeres erode and this shortening is directly linked to aging. Yet, despite the fact that laboratory mice have lifetimes roughly $1/30^{th}$ that of a human, murine telomeres are approximately 5–10 times longer than their human counterparts. The regulation of telomeres is therefore quite different between species and using the mouse to understand human

telomere biology in HSC is fraught with concerns. The extent to which telomere biology overlaps between the mouse and human has been well highlighted by introducing genetic lesions found in humans with telomere regulation deficiencies into mouse models.

Telomere length is regulated by several multi-subunit complexes including Telomerase-regulatory factor-1 and -2 (TRF-1 and TRF-2) and telomerase, comprised of telomerase reverse transcriptase (TERT), telomerase RNA template (TERC) and Dyskerin (DKC1). Humans do have naturally occurring mutations in telomere regulators with the most notable disease being Dyskeratosis congenita (DC). DC is an inherited disorder that is characterized by bone marrow failure and mucocutaneous abnormalities such as abnormal pigmentation, leukoplakia and nail dystrophy. Cells from the vast majority of DC patients display shorter telomeres, diminished telomerase activity and telomere length inversely correlates with disease severity. The most common mutation found in DC occurs in the *DKC1* gene (~30% of cases), which is a pseudouridine synthase that regulates ribosome biogenesis as well as stabilizes the RNA component of telomerase.[78] DC patients less frequently display mutations in telomerase complex constituents, *TCAB1, NOP10* and *NHP2*, and the gene encoding TIN2 (i.e. *TINF2*), a component of the TRF-1 complex that regulates telomerase activity. Some of the genetic lesions in human have been introduced into mice. For example, conditional deletion of *TRF1*, which complexes with TIN2 to regulate telomerase activity, in the murine hematopoietic system recapitulates the bone marrow failure phenotype seen in patients with DC and *TRF1* mutation.[79] The correlation is less compelling with other lesions. Genetic ablation of *mTerc* results in some of the abnormalities seen in DC patients with *TERC* mutations, however these phenotypes only manifest in offspring that are the product of numerous generations of *mTerc*$^{-/-}$ interbreeding.[80] The lack of overt hematological phenotype in the first generation *mTert* or *mTerc* knockout mice may be due to the length of existing telomeres in the mouse, but does raise concern that mouse models may not perfectly mimic the principles of telomere participation in human disease and human aging.

Concluding remarks

The mouse has been an invaluable tool for defining the HSC, establishing principles by which it is regulated and enabling the development of assays to measure HSC behavior. The insights gained from the mouse have largely proven true in the human in both the larger issue of stem cell function and in the regulation of stem cell localization, however, direct correspondence is often lacking in specific key areas. These areas can be viewed as reflective of the dramatic difference in the lifespan of the mouse relative to the human. The replicative rate of stem cells, their relative contribution to descendent cells, their cell cycle governance and the means by which they undergo DNA repair all appear to be distinctive in the human. Therefore, caveat emptor; the mouse is a guide but not an invariant source of the truth for human biology. Where it is most reliable is in events not linked to preservation of the HSC over decades of life.

References

1. Kennedy M, et al. A common precursor for primitive erythropoiesis and definitive haematopoiesis. Nature. 1997; 386:488–493. [PubMed: 9087406]
2. Choi K, Kennedy M, Kazarov A, Papadimitriou JC, Keller G. A common precursor for hematopoietic and endothelial cells. Development. 1998; 125:725–732. [PubMed: 9435292]
3. Palis J, Robertson S, Kennedy M, Wall C, Keller G. Development of erythroid and myeloid progenitors in the yolk sac and embryo proper of the mouse. Development. 1999; 126:5073–5084. [PubMed: 10529424]

4. Samokhvalov IM, Samokhvalova NI, Nishikawa S. Cell tracing shows the contribution of the yolk sac to adult haematopoiesis. Nature. 2007; 446:1056–1061. [PubMed: 17377529]
5. Medvinsky A, Dzierzak E. Definitive hematopoiesis is autonomously initiated by the AGM region. Cell. 1996; 86:897–906. [PubMed: 8808625]
6. Ivanovs A, et al. Highly potent human hematopoietic stem cells first emerge in the intraembryonic aorta-gonad-mesonephros region. J Exp Med. 2011; 208:2417–2427. [PubMed: 22042975]
7. Ramalho-Santos M, Willenbring H. On the origin of the term “stem cell”. Cell Stem Cell. 2007; 1:35–38. [PubMed: 18371332]
8. McCulloch EA, Till JE. The radiation sensitivity of normal mouse bone marrow cells, determined by quantitative marrow transplantation into irradiated mice. Radiat Res. 1960; 13:115–125. [PubMed: 13858509]
9. Becker AJ, Mc CE, Till JE. Cytological demonstration of the clonal nature of spleen colonies derived from transplanted mouse marrow cells. Nature. 1963; 197:452–454. [PubMed: 13970094]
10. Till JE, McCulloch EA, Siminovitch L. A Stochastic Model of Stem Cell Proliferation, Based on the Growth of Spleen Colony-Forming Cells. Proc Natl Acad Sci U S A. 1964; 51:29–36. [PubMed: 14104600]
11. Spangrude GJ, Heimfeld S, Weissman IL. Purification and characterization of mouse hematopoietic stem cells. Science. 1988; 241:58–62. [PubMed: 2898810]
12. Okada S, et al. Enrichment and characterization of murine hematopoietic stem cells that express c-kit molecule. Blood. 1991; 78:1706–1712. [PubMed: 1717068]
13. Kiel MJ, Yilmaz OH, Iwashita T, Terhorst C, Morrison SJ. SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. Cell. 2005; 121:1109–1121. [PubMed: 15989959]
14. Osawa M, Hanada K, Hamada H, Nakauchi H. Long-term lymphohematopoietic reconstitution by a single CD34-low/negative hematopoietic stem cell. Science. 1996; 273:242–245. [PubMed: 8662508]
15. Rappold I, et al. Functional and phenotypic characterization of cord blood and bone marrow subsets expressing FLT3 (CD135) receptor tyrosine kinase. Blood. 1997; 90:111–125. [PubMed: 9207445]
16. van der Wath RC, Wilson A, Laurenti E, Trumpp A, Lio P. Estimating dormant and active hematopoietic stem cell kinetics through extensive modeling of bromodeoxyuridine label-retaining cell dynamics. PloS one. 2009; 4:e6972. [PubMed: 19771180]
17. Udomsakdi C, Eaves CJ, Sutherland HJ, Lansdorp PM. Separation of functionally distinct subpopulations of primitive human hematopoietic cells using rhodamine-123. Exp Hematol. 1991; 19:338–342. [PubMed: 1709107]
18. Goodell MA, Brose K, Paradis G, Conner AS, Mulligan RC. Isolation and functional properties of murine hematopoietic stem cells that are replicating in vivo. J Exp Med. 1996; 183:1797–1806. [PubMed: 8666936]
19. Kamel-Reid S, Dick JE. Engraftment of immune-deficient mice with human hematopoietic stem cells. Science. 1988; 242:1706–1709. [PubMed: 2904703]
20. Larochelle A, et al. Human and rhesus macaque hematopoietic stem cells cannot be purified based only on SLAM family markers. Blood. 2011; 117:1550–1554. [PubMed: 21163926]
21. Zhou S, et al. The ABC transporter Bcrp1/ABCG2 is expressed in a wide variety of stem cells and is a molecular determinant of the side-population phenotype. Nat Med. 2001; 7:1028–1034. [PubMed: 11533706]
22. Berardi AC, Wang A, Levine JD, Lopez P, Scadden DT. Functional isolation and characterization of human hematopoietic stem cells. Science. 1995; 267:104–108. [PubMed: 7528940]
23. Abkowitz JL, Catlin SN, McCallie MT, Guttorp P. Evidence that the number of hematopoietic stem cells per animal is conserved in mammals. Blood. 2002; 100:2665–2667. [PubMed: 12239184]
24. Spangrude GJ, Johnson GR. Resting and activated subsets of mouse multipotent hematopoietic stem cells. Proc Natl Acad Sci U S A. 1990; 87:7433–7437. [PubMed: 1977160]

25. Passegue E, Wagers AJ, Giuriato S, Anderson WC, Weissman IL. Global analysis of proliferation and cell cycle gene expression in the regulation of hematopoietic stem and progenitor cell fates. J Exp Med. 2005; 202:1599–1611. [PubMed: 16330818]
26. Cheshier SH, Morrison SJ, Liao X, Weissman IL. In vivo proliferation and cell cycle kinetics of long-term self-renewing hematopoietic stem cells. Proc Natl Acad Sci U S A. 1999; 96:3120–3125. [PubMed: 10077647]
27. Bradford GB, Williams B, Rossi R, Bertoncello I. Quiescence, cycling, and turnover in the primitive hematopoietic stem cell compartment. Exp Hematol. 1997; 25:445–453. [PubMed: 9168066]
28. Kiel MJ, et al. Haematopoietic stem cells do not asymmetrically segregate chromosomes or retain BrdU. Nature. 2007; 449:238–242. [PubMed: 17728714]
29. Wilson A, et al. Dormant and self-renewing hematopoietic stem cells and their niches. Ann N Y Acad Sci. 2007; 1106:64–75. [PubMed: 17442778]
30. Foudi A, et al. Analysis of histone 2B-GFP retention reveals slowly cycling hematopoietic stem cells. Nat Biotechnol. 2009; 27:84–90. [PubMed: 19060879]
31. Mahmud N, et al. The relative quiescence of hematopoietic stem cells in nonhuman primates. Blood. 2001; 97:3061–3068. [PubMed: 11342431]
32. Catlin SN, Busque L, Gale RE, Guttorp P, Abkowitz JL. The replication rate of human hematopoietic stem cells in vivo. Blood. 2011; 117:4460–4466. [PubMed: 21343613]
33. Jacobson LO, Simmons EL, Marks EK, Eldredge JH. Recovery from radiation injury. Science. 1951; 113:510–511. [PubMed: 14828383]
34. Lord BI. The architecture of bone marrow cell populations. Int J Cell Cloning. 1990; 8:317–331. [PubMed: 2230283]
35. Dexter TM, Allen TD, Lajha LG. Conditions controlling the proliferation of hemopoietic stem cells in vitro. J Cell Physiol. 1977; 91:335–344. [PubMed: 301143]
36. Schofield R. The relationship between the spleen colony-forming cell and the haemopoietic stem cell. Blood Cells. 1978; 4:7–25. [PubMed: 747780]
37. Zhang J, et al. Identification of the haematopoietic stem cell niche and control of the niche size. Nature. 2003; 425:836–841. [PubMed: 14574412]
38. Calvi LM, et al. Osteoblastic cells regulate the haematopoietic stem cell niche. Nature. 2003; 425:841–846. [PubMed: 14574413]
39. Yamazaki S, et al. Nonmyelinating Schwann cells maintain hematopoietic stem cell hibernation in the bone marrow niche. Cell. 147:1146–1158. [PubMed: 22118468]
40. Butler JM, et al. Endothelial cells are essential for the self-renewal and repopulation of Notch-dependent hematopoietic stem cells. Cell Stem Cell. 2010; 6:251–264. [PubMed: 20207228]
41. Doan PL, et al. Tie2(+) bone marrow endothelial cells regulate hematopoietic stem cell regeneration following radiation injury. Stem Cells. 2013; 31:327–337. [PubMed: 23132593]
42. Mendez-Ferrer S, Lucas D, Battista M, Frenette PS. Haematopoietic stem cell release is regulated by circadian oscillations. Nature. 2008; 452:442–447. [PubMed: 18256599]
43. Winkler IG, et al. Bone marrow macrophages maintain hematopoietic stem cell (HSC) niches and their depletion mobilizes HSCs. Blood. 116:4815–4828. [PubMed: 20713966]
44. Chow A, et al. Bone marrow CD169+ macrophages promote the retention of hematopoietic stem and progenitor cells in the mesenchymal stem cell niche. J Exp Med. 2011; 208:261–271. [PubMed: 21282381]
45. Kollet O, et al. Osteoclasts degrade endosteal components and promote mobilization of hematopoietic progenitor cells. Nat Med. 2006; 12:657–664. [PubMed: 16715089]
46. Semerad CL, et al. G-CSF potently inhibits osteoblast activity and CXCL12 mRNA expression in the bone marrow. Blood. 2005; 106:3020–3027. [PubMed: 16037394]
47. Katayama Y, et al. Signals from the Sympathetic Nervous System Regulate Hematopoietic Stem Cell Egress from Bone Marrow. Cell. 2006; 124:407–421. [PubMed: 16439213]
48. Naveiras O, et al. Bone-marrow adipocytes as negative regulators of the haematopoietic microenvironment. Nature. 2009; 460:259–263. [PubMed: 19516257]

49. Mendez-Ferrer S, et al. Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. Nature. 466:829–834. [PubMed: 20703299]
50. Ding L, Saunders TL, Enikolopov G, Morrison SJ. Endothelial and perivascular cells maintain haematopoietic stem cells. Nature. 2012; 481:457–462. [PubMed: 22281595]
51. Ferraro F, et al. Diabetes impairs hematopoietic stem cell mobilization by altering niche function. Science translational medicine. 3:104ra101.
52. Ma Q, Jones D, Springer TA. The chemokine receptor CXCR4 is required for the retention of B lineage and granulocytic precursors within the bone marrow microenvironment. Immunity. 1999; 10:463–471. [PubMed: 10229189]
53. Kawabata K, et al. A cell-autonomous requirement for CXCR4 in long-term lymphoid and myeloid reconstitution. Proc Natl Acad Sci U S A. 1999; 96:5663–5667. [PubMed: 10318941]
54. Shen H, et al. CXCR-4 desensitization is associated with tissue localization of hemopoietic progenitor cells. J Immunol. 2001; 166:5027–5033. [PubMed: 11290783]
55. Craddock CF, Nakamoto B, Andrews RG, Priestley GV, Papayannopoulou T. Antibodies to VLA4 integrin mobilize long-term repopulating cells and augment cytokine-induced mobilization in primates and mice. Blood. 1997; 90:4779–4788. [PubMed: 9389694]
56. North TE, et al. Prostaglandin E2 regulates vertebrate haematopoietic stem cell homeostasis. Nature. 2007; 447:1007–1011. [PubMed: 17581586]
57. Adams GB, et al. Haematopoietic stem cells depend on Galpha(s)-mediated signalling to engraft bone marrow. Nature. 2009; 459:103–107. [PubMed: 19322176]
58. Adams GB, et al. Therapeutic targeting of a stem cell niche. Nat Biotechnol. 2007; 25:238–243. [PubMed: 17237769]
59. Fulzele K, et al. Myelopoiesis is regulated by osteocytes through Gsalpha-dependent signaling. Blood. 2013; 121:930–939. [PubMed: 23160461]
60. Walkley CR, et al. A microenvironment-induced myeloproliferative syndrome caused by retinoic acid receptor gamma deficiency. Cell. 2007; 129:1097–1110. [PubMed: 17574023]
61. Zimmer SN, et al. Crebbp haploinsufficiency in mice alters the bone marrow microenvironment, leading to loss of stem cells and excessive myelopoiesis. Blood. 2011; 118:69–79. [PubMed: 21555743]
62. Raaijmakers MH, et al. Bone progenitor dysfunction induces myelodysplasia and secondary leukaemia. Nature. 464:852–857. [PubMed: 20305640]
63. Kohler A, et al. Altered cellular dynamics and endosteal location of aged early hematopoietic progenitor cells revealed by time-lapse intravital imaging in long bones. Blood. 2009; 114:290–298. [PubMed: 19357397]
64. Vas V, Senger K, Dorr K, Niebel A, Geiger H. Aging of the microenvironment influences clonality in hematopoiesis. PloS one. 2012; 7:e42080. [PubMed: 22879906]
65. Marcondes AM, et al. Dysregulation of IL-32 in myelodysplastic syndrome and chronic myelomonocytic leukemia modulates apoptosis and impairs NK function. Proc Natl Acad Sci U S A. 2008; 105:2865–2870. [PubMed: 18287021]
66. Blau O, et al. Mesenchymal stromal cells of myelodysplastic syndrome and acute myeloid leukemia patients have distinct genetic abnormalities compared with leukemic blasts. Blood. 2011; 118:5583–5592. [PubMed: 21948175]
67. Pang WW, et al. Human bone marrow hematopoietic stem cells are increased in frequency and myeloid-biased with age. Proc Natl Acad Sci U S A. 2011; 108:20012–20017. [PubMed: 22123971]
68. Kuranda K, et al. Age-related changes in human hematopoietic stem/progenitor cells. Aging cell. 2011; 10:542–546. [PubMed: 21418508]
69. Liang Y, Van Zant G, Szilvassy SJ. Effects of aging on the homing and engraftment of murine hematopoietic stem and progenitor cells. Blood. 2005; 106:1479–1487. [PubMed: 15827136]
70. Kollman C, et al. Donor characteristics as risk factors in recipients after transplantation of bone marrow from unrelated donors: the effect of donor age. Blood. 2001; 98:2043–2051. [PubMed: 11567988]

71. Morrison SJ, Wandycz AM, Akashi K, Globerson A, Weissman IL. The aging of hematopoietic stem cells [see comments]. Nat Med. 1996; 2:1011–1016. [PubMed: 8782459]

72. Cho RH, Sieburg HB, Muller-Sieburg CE. A new mechanism for the aging of hematopoietic stem cells: aging changes the clonal composition of the stem cell compartment but not individual stem cells. Blood. 2008; 111:5553–5561. [PubMed: 18413859]

73. Beerman I, et al. Functionally distinct hematopoietic stem cells modulate hematopoietic lineage potential during aging by a mechanism of clonal expansion. Proc Natl Acad Sci U S A. 2010; 107:5465–5470. [PubMed: 20304793]

74. Notta F, et al. Isolation of single human hematopoietic stem cells capable of long-term multilineage engraftment. Science. 2011; 333:218–221. [PubMed: 21737740]

75. Chambers SM, et al. Aging hematopoietic stem cells decline in function and exhibit epigenetic dysregulation. PLoS biology. 2007; 5:e201. [PubMed: 17676974]

76. Rossi DJ, et al. Deficiencies in DNA damage repair limit the function of haematopoietic stem cells with age. Nature. 2007; 447:725–729. [PubMed: 17554309]

77. Rube CE, et al. Accumulation of DNA damage in hematopoietic stem and progenitor cells during human aging. PloS one. 2011; 6:e17487. [PubMed: 21408175]

78. Mitchell JR, Wood E, Collins K. A telomerase component is defective in the human disease dyskeratosis congenita. Nature. 1999; 402:551–555. [PubMed: 10591218]

79. Beier F, Foronda M, Martinez P, Blasco MA. Conditional TRF1 knockout in the hematopoietic compartment leads to bone marrow failure and recapitulates clinical features of dyskeratosis congenita. Blood. 2012; 120:2990–3000. [PubMed: 22932806]

80. Rudolph KL, et al. Longevity, stress response, and cancer in aging telomerase-deficient mice. Cell. 1999; 96:701–712. [PubMed: 10089885]

Table 1

The Hematopoietic Stem Cell: Mouse vs. Human

	Mouse	Human
Approximate Size	25–35 g	62–88 kg
Approximate Lifespan	2–2.5 yrs	70 yrs
Development stages		
Hemanigioblast	Yes	Yes
Yolk Sac	Yes	Yes
Aorta-Gonad-Mesoderm (AGM)	Yes	Yes
Extra-embryonic tissue	Yes	Yes
HSC Identity (Lineage Low)		
CD117 (c-Kit)	+	+
Sca-1	+	−
SLAM ($CD150^+$, $CD48^-$, $CD244^-$)	+	−
CD34, CD49f	−	+
Efflux dyes	+	+/−
Growth Properties		
Replication rates	~ 2.5 weeks	~ 40 weeks
Active:Quiescent HSCs	1.46	0.116
HSC mobilization		
G-CSF	Yes	Yes
CXCR4-CXCL12	Yes	Yes
α4β1 Integrin (VLA4)	Yes	Yes
HSC Aging		
Myeloid:Lymphoid	Increases	Increases
DNA-Damage Response (DDR)	NHEJ	P53-mediated
Relative Telomere Length	5–10	1