Hematopoietic Stem Cells and the Aging Hematopoietic System

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The etiology of the age-associated pathophysiological changes of the hematopoietic system including the onset of anemia, diminished adaptive immune competence, and myelogenous disease development are underwritten by the loss of normal homeostatic control. As tissue and organ homeostasis in adults is primarily mediated by the activity of stem and progenitor cells, it has been suggested that the imbalances accompanying aging of the hematopoietic system may stem from alterations in the prevalence and/or functional capacity of hematopoietic stem cells (HSCs) and progenitors. In this review, we examine evidence implicating a role for stem cells in the aging of the hematopoietic system, and focus on the mechanisms suggested to contribute to stem cell aging.

Aging is one of inevitabilities of life to which no one is immune. Essentially all aspects of our physiology and phenotype undergo slow but steady change as we age. In contrast to aging of other organs such as the skin and hair, which manifest readily observable physiological decline, aging within the hematopoietic system is generally more subtle—hematopoietic failure is exceedingly rare, and almost always associated with a specific pathological condition. Nonetheless, detailed studies have clearly documented substantial changes in function of several hematopoietic lineages as we age, perhaps best exemplified in the decreased competence of the adaptive immune system observed in the elderly.1

Hematopoietic stem cells (HSCs) function throughout life to generate all of the effector cells of the hematopoietic system. HSCs are imbued with enormous developmental potential; even a single stem cell can reconstitute the entire blood system.2 The extraordinary developmental potential of HSCs is achieved by their unique ability to both self-renew to ensure that stem cell reserves are maintained over time, and to differentiate to give rise to downstream effector cells. HSCs have emerged as the model system for studying the effects of aging on stem cell biology since they can be purified to near homogeneity, and can be quantitatively and qualitatively assayed with high resolution. In this review we discuss the role of HSCs in the aging of the hematopoietic system (Figure 1), focusing on underlying mechanisms. Since much of what we know about aging and HSC function is founded on studies using mice, we will primarily restrict our discussion to this system.

Hematopoietic Stem Cell Ontogeny

Although most studies of aging focus primarily on the pathophysiological changes that arise late in life, we believe that a consideration of ontogeny is important when thinking about aging within adult stem cell compartments as it could be argued that onset of aging of such cells commences at the time at which the stem cell pool is maintained solely by self-renewal, and subsequent to input from more primitive progenitors. In mice, hematopoiesis begins at embryonic day 7 (E7.0) with the emergence of primitive progenitors in the yolk sac (YS) capable of generating erythrocytes that are essential for the oxygenation of the rapidly growing embryo.3,4 Subsequent to the yolk sac, hematopoiesis takes place in the aorta-gonad mesonephros (AGM), which has been suggested to be the site where definitive HSCs arise.5 Stem cells then seed the fetal liver, thymus, spleen, and ultimately the bone marrow (BM). While these former sites are all colonized by migrating HSCs, the developmental relationship between the primary sites of hematopoiesis and the emergence of definitive HSCs is still controversial.6 Although YS progenitors were originally believed to contribute to definitive hematopoiesis, it is now known that definitive HSCs arise de novo in the AGM. Other studies have suggested that definitive HSCs arise from YS progenitors in a self-renewing population.7 Thus, a critical role of hematopoietic stem cell ontogeny is to ensure the maintenance of self-renewing stem cell populations to provide a reserve source of HSCs throughout life.
poiesis, several lines of evidence from experiments using chicken-quail chimeras suggested that this might not be the case. Surprisingly, however, recent in vivo lineage-tracing experiments, using mice heterozygous for Runx1, demonstrated that YS hematopoiesis can contribute to the definitive pool of adult HSCs, although the extent of this contribution is yet to be determined.

HSCs arising in the developing fetus differ from the adult not only in their location but also in several key properties, including their cell surface phenotype. Moreover, in contrast to adult HSCs, which reside primarily in the quiescent (G0) phase of the cell cycle, fetal HSCs proliferate rapidly, until about 3 weeks after birth, consistent with their role in the establishment of the hematopoietic compartment.

Functionally, fetal HSCs produce particular blood cell subtypes that are not produced by adult HSCs such as γδ T cells and the B1a B cells, suggesting that the embryonic to neonatal transition already includes some limitations on differentiation potency. The ability of human HSCs to generate primitive progenitors in vitro was reported to be higher in the fetal liver compared to umbilical cord blood, and was further diminished when HSCs were isolated from adult BM. It seems likely that many of the functional changes that accompany the transition from fetal to post-natal stem cell activity arise as a consequence of the changing developmental demands of fetal versus adult hematopoiesis. The transition from embryonic to adult hematopoiesis is accompanied by profound differences in regulatory control. For example, the

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**Figure 1** Aging within the hematopoietic system. Schematic representation of the hematopoietic hierarchy, with noted consequences of aging on hematopoietic stem and progenitor cells, and mature effector cells. It should be noted that the hierarchy presented is a simplified model of hematopoietic differentiation. Black block arrows pointing up or down represent an increase or a decrease, respectively. The cell surface phenotype of hematopoietic stem cells is noted for mice and man. n/c, not changed.
transcription factor Sox17 is required for the maintenance and function of fetal HSCs, but not for the adult, while Gfi-1 and Etv6 appear to be exclusively required for sustaining adult HSCs.21,26 Such a dichotomy of dependence on divergent transcriptional regulators reveals a fundamental alteration in the molecular mechanisms regulating stem cell identity during ontogeny, that appears to enable the transition from initial establishment of the hematopoietic system, to their role in adult homeostasis and long-term maintenance. Whether or not such molecular regulators represent potential targets for the re-establishment of embryonic potential onto adult HSCs in a manner akin to the reprogramming of somatic cells into induced pluripotent stem cells (iPS)18 has yet to be tested. Nonetheless, the fact that divergent molecular regulators can function at the level of stem cells to affect developmental fate during early stages of ontogeny also suggests that at least some of the functional changes that accompany the transition to old age may similarly be regulated and not simply stochastic.

**Hematopoietic Stem Cell Reserves During Aging**

A commonly held belief about how aging within stem cell compartments impacts tissue integrity is that stem cell reserves progressively diminish with age and eventually cannot meet homeostatic demands for cell turnover. Indeed, such a mechanism appears to contribute to one of the most obvious phenotypes of aging, hair graying, which has been reported to be associated with gradual loss of melanocyte stem cells in the hair follicle bulge with age.19 On the other hand, epidermal stem cells, which reside in proximity to melanocyte stem cells, exhibit age-dependent diminished activity but appear to maintain their numbers.20 The effect of aging on the HSC compartment is more complex and depends on variables such as the type of assay used to evaluate stem cell numbers and the genetic strain of mouse assayed. Early experiments demonstrated that whole BM from old C57BL/6 mice was superior to young marrow with regard to the ability to competitively reconstitute the erythroid compartment of irradiated, or unconditioned W/Wv recipients.21 This observation was later confirmed and elaborated by quantification of additional lineages in experiments that showed a two- to threefold higher potency of the total BM cells from older mice.10,22 These counterintuitive results suggested that the bulk stem cell potential in mice is not diminished but rather increased with age, regardless of per-cell ability. Maintenance of HSCs potency with advancing age is in line with serial transplantation experiments, which demonstrated that HSCs possess the ability to function through at least 15 to 50 normal life spans.23 One limitation to whole BM transplantation experiments is that they cannot discriminate between enhanced per-cell potency of HSCs, or an age-dependent increase in their frequency within the BM. Toward measuring HSCs frequencies, statistical estimations,24 limit dilution experiments,10,25 and in vitro culturing assays22 have all suggested an approximately twofold increase in the number of HSCs in the BM of old versus young C57BL/6 mice, although differences between genetic backgrounds have been observed.21,26 This latter point was elegantly demonstrated in experiments in which aggregation chimeras of two strains (DBA/2 and C57BL/6) initially revealed equivalent blood cell production that shifted to favor C57BL/6 chimerism with age,27 illuminating the existence of genetic differences capable of sustaining HSC activity.

HSCs can be prospectively isolated by cell surface phenotype and functionally validated. Divergent criteria have emerged over the years that more or less rigorously define the immunophenotypic identity of HSCs, and therefore one must critically evaluate divergent studies with regard to the exact cell surface phenotype used. For example, the most commonly used strategy for enriching HSC activity by surface phenotype (c-kit+Lineage−Sca-1+ [KLS]) does indeed enrich for stem cell activity from total BM yet does not distinguish HSCs from the much more numerous multi-potent progenitor subsets that constitute the majority of the KLS fraction. The use of additional selection criteria such as CD34+, flk2, and CD48+CD150+ has allowed for much better resolution between stem and progenitor cells in recent years.2,26-31 Various studies, using comparable but not uniform methods, suggested that HSCs increase in frequency with age by a factor of 6- to 17-fold.10,32-35 Interestingly, quantitation by immunophenotype in C57BL/6 aging mice is consistently much higher than the aforementioned functional estimates have suggested. Indeed, competitive transplantation studies using purified stem cells have shown that the per-cell activity of HSCs from old mice was substantially reduced compared to young.10,33,36 Intriguingly, sorted HSCs from old mice were functionally shown to establish a larger stem cell compartment in primary transplant recipients,33,34 suggesting that the steady-state expansion of the stem cell pool with age results from a cell autonomous increased self-renewal capacity. Taken together, these studies suggest that while the per-cell activity of HSCs diminishes with age, their total functional output within the BM is sustained by a significant increase in their numbers.

**Functional Changes in HSCs With Age**

Studies have shown that blood cell composition changes with age. Most notably, while lymphopoiesis is robust in the young, it declines with age in mice10,37 and humans.1 And while thymic involution and an overabundance of lymphoid cells competing for limiting cytokine resources clearly factors into lymphopoietic decline,1 studies have shown that lymphopoietic decline (particularly B cell) is also an intrinsic property of HSC aging.10,33 Interestingly, while lymphoid potential diminishes, myeloid potential (particularly granulopoiesis) is maintained, or even increased during aging. This skewing of lineage potential (from lymphopoiesis towards myelopoiesis) was evident even when small numbers of stem cells were transplanted.10,33 This altered lineage potential was evident at the level of the oligo-potent myeloid13 and lym-
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Mechanisms Underlying HSC Aging

The molecular mechanisms underlying HSC aging are poorly understood. As noted, global gene expression profiling of HSCs isolated from young and old mice revealed coordinated regulation of groups of lineage-specification genes consistent with the functional changes observed as HSCs age. Moreover, age-dependent regulation of genes involved in chroma-
tin remodeling and coordinated regulation along higher order chromosome structures suggests that higher order chromatin dynamics may be involved in HSC aging. DNA methylation and histone acetylation are the prominent long-term epigenetic modifications that regulate cellu-
lar differentiation. Ex vivo studies of human cells suggested that histone deacetylase (HDAC) inhibitors might delay the loss of stem cell potential in vitro and enhance engraftment abil-
ity. In mice, the manipulations of polycomb genes dram-
atically influence the fate of stem cells. Overexpression of Ezh2 functionally extended the potential of HSCs through serial transplantation. Bmi1 deficiency demonstrated its es-
sential role in the long-term maintenance of the stem cell compartment in vivo, and enforced expression of Bmi1 dramatically promoted both in vitro self-renewal and the repopulation activity of HSCs. Interestingly, Bmi1 has also been implicated in the maintenance of neuronal stem cells and to function in leukemic stem cells. A possible common mechanism for these pathways is the inhibition of p16^ink4a, p19^arf (growth-suppressing genes) by Bmi1 together with Ezh2. Recently, the role of de novo DNA methylation was evaluated in HSCs by conditional depletion of both Dnmt3a and Dnmt3b. Interestingly, these experiments revealed an essential role for de novo DNA methylation in stem cell main-
tenance but not in differentiation potential. This was partic-
ularly interesting since the authors demonstrated that the overall genomic methylation substantially increases as hema-
topoietic differentiation progresses, while global methylation was below detection levels in HSCs themselves, thus high-
lighting a paradox of dependence on de novo DNA methyl-
ation in hematopoietic differentiation versus self-renewal of HSCs. These results suggest that perhaps only a few key genes require de novo DNA methylation by Dnmt3a and Dnmt3b in HSCs to maintain self-renewal capacity, while hematopoietic differentiation is essentially Dnmt3a- and Dnmt3b-independent in spite of normally increasing amounts of global DNA methylation.

Telomere length is perhaps the most well-known “molecular clock” of cell division with a well-documented role in maintaining stem cell function. In humans, mutations in the telomerase RNA catalytic components, or in associated proteins lead to pancytopenia and BM failure in patients with aplastic anemia and/or dyskeratosis congenita through stem and progenitor cell defects. In mice, genetic ablation of telomerase activity and telomere attrition diminishes HSC repopulation potential in an age-dependent manner, and ultimately limits HSC replicative capacity in serial transplantation assays. Interestingly, ectopic overexpression of the catalytic component of telomerase in HSCs was insufficient to extend their serial transplant capacity, suggesting that telomere-length independent mechanisms may ultimately limit the replicative capacity of HSCs.

Reactive oxygen species (ROS) and oxidative damage is thought to be a major factor contributing to the aging of many cell types. Interestingly, HSCs may be somewhat protected from this type of damage due to their position in the postupe-
lated bone marrow niche, which is thought to be hy-
poxic, although it should be noted that the precise loca-
tion and cellular composition of the stem cell niche has yet to be convincingly determined. Moreover, the fact that HSCs cycle infrequently and have a concomitantly low metabolic activity may also serve to limit their exposure to oxidative stress. Another protective mechanism active in HSCs is achieved through high expression of ATP-binding cassette (ABC) transporters that pump xenobiotic and hazardous compounds out of the cell. On the other hand, HSCs ex-
hibited a blunted potency in the absence of ATM and FoxO transcrip-
tion factors that appeared to be mediated by oxidative stress. High levels of ROS in the Atm-deficient mice resulted in activation of p38 mitogen-activated protein ki-
nase (MAPK) and induced proliferation, which was prevented by either anti-oxidant treatment or specific p38 inhibitors.63 Similarly, the stem cell deficits observed in mice with triple deficiency of Foxo1, 3, and 4 were at least in part reverted by antioxidant treatment.62 In a related story, mice-deficient in Foxo3a showed normal hematopoietic development but exhibited multiple stem cell deficiencies with advanced age.51 Foxo3 deficiency was further shown to affect HSCs through ATM in part and to involve the p53/p21 pathway.40 Together, these studies identify intracellular management of ROS levels as an important mechanism contributing to the preservation of HSC reserves and function during aging.

Accumulation of DNA damage is thought to be a central mechanism contributing to cellular decline. Indeed the importance of genomic maintenance for preserving stem cell function has been demonstrated in numerous studies evaluating HSC function in mice deficient in divergent DNA repair pathways, including FancD1/Brca2,65 Msh2,66 Lig1,67 Rad50,68 mTR, XPD, and Ku80.40 These studies suggest that while steady-state hematopoiesis can often be maintained in the absence of key DNA repair proteins, the capacity of HSCs to respond to acute stress is severely impaired in the absence of these factors. Interestingly, immunohistochemical examination of single highly purified HSCs from young and old mice to assess DNA damage with age revealed that stem cells from old mice contained considerably more DNA damage than their young counterparts, as indicated by an age-dependent accumulation of γH2AX foci.40 Importantly, the examination of downstream multi-potent and oligo-potent progenitors from old mice exhibited substantially less γH2AX foci, suggesting that age-dependent DNA damage accrual was restricted to the stem cell compartment.40 These findings suggest a mechanism whereby stem cells themselves may serve as the reservoir for the acquisition of mutational events required for disease pathogenesis, or oncogenic transformation. This prospect is particularly disquieting in the context of stem cell biology since in contrast to damage accrual in somatic cells, the self-renewal and differentiation potential of stem cells affords the opportunity to potentiate the impact of genetic damage throughout the hematopoietic hierarchy as lesions arising in the stem cell compartment can be propagated through self-renewing progeny, and conveyed to downstream progenitors. Considering the long lifespan of humans, genetic lesions accumulated in the stem cell compartment over time may play a significant role in the origins of a variety of pathologies in the elderly. The direct assessment of DNA damage during stem cell aging in humans is therefore of clear interest.

### Extrinsic Control of HSC Aging

The functional capacity of stem cells in the aging organism is determined both by intrinsic and extrinsic factors. While our ultimate goal is to understand the final outcome, it is important to discriminate between the different mechanisms that influence HSC activity. An early study using subcutaneous implantation of bones from young or old mice, demonstrated decreased repopulation of young cells into the old bones grafted onto young mice, which suggested a less favorable attraction, retention, or viable support for colonization in the old niches.69 This complicated experiment controlled for additional factors such as systemic cytokines by reciprocally implanting young bones onto old mice and observing a better performance in terms of young donor tissue colonization by the old recipient cells. These experiments suggest that the aged BM environment is less capable of supporting stem or progenitor cell function. Similarly, in vitro long-term BM cultures on stromal cells derived from either young or old mice has demonstrated a reduced ability of the old stroma to support hematopoietic progenitor activity.70

The influence of age on stem cell trafficking has also been addressed in a recent study, which revealed that young cells had a twofold greater capacity to migrate into the BM than the cells from old mice.23 This study suggested that the older milieu itself is less favorable for retaining cells within the BM. More evidence that aging is associated with a change in the way in which HSCs interact with BM microenvironment was revealed in a study that reported a general increase in the mobilization of HSCs and progenitors in old mice due to reduced adherence with stroma cells.71

A striking example of how HSC-microenvironment interactions can influence the function of the hematopoietic system comes from studies of RARγ-deficient mice, which develop a myeloproliferative disease (MPD) that cannot be rescued by transplantation with normal BM; whereas RARγ-deficient BM cells transplanted into wild-type recipients differentiate normally and do not develop a MPD.72 These striking results demonstrate that a profound hematopoietic pathology may be entirely derived through stromal cell instruction. It is important to contrast this scenario with the clinical success of BM transplantation to correct many other life-threatening diseases.

### Conclusion

Strategies aimed at diminishing the impact of infectious diseases, combined with advances in medicine and sanitation, have succeeded in increasing the average life expectancy substantially. Indeed, population demographics indicate that the percentage of elderly adults is at a historical high, and continues to climb. However, this increase has come at a cost as age-related degenerative and malignant conditions and associated morbidities are increasingly prevalent. The need to develop therapeutic strategies aimed at ameliorating pathophysiological conditions in the elderly is thus quite urgent. The enormous therapeutic potential of stem cells in regenerative medicine has evoked great excitement for the development of strategies aimed at treating the types of degenerative conditions prevalent in the elderly. Characterization of how stem cell aging contributes to age-associated pathophysiological decline is the critical first step towards achieving these goals, as such research should be able to identify the mechanisms underlying stem cell functional decline and at the same time inform strategies for intervention.
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