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Hematopoietic Stem Cell Aging: Mechanism and Consequence

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Abstract

Advancing age is frequented by the onset of a variety of hematological conditions characterized by diminished homeostatic control of blood cell production. The fact that upstream hematopoietic stem and progenitor cells are obligate mediators of homeostatic control of all blood lineages, has implicated the involvement of these cells in the pathophysiology of these conditions. Indeed, evidence from our group and others has suggested that two of the most clinically significant age-associated hematological conditions, namely, the diminution of the adaptive immune system, and the elevated incidence of myeloproliferative diseases, have their origin in cell autonomous changes in the functional capacity of hematopoietic stem cells.

Introduction

In the adult, homeostatic control of tissues is mediated by tissue-specific stem cells, which give rise to the differentiated cell types comprising of diverse tissues and organs. Once believed to be restricted to tissues with high turn-over rates such as the blood, gut and skin, stem cells are now thought to be involved in controlling homeostasis in most tissues, including those with low turn-over rates, as well as those that were once thought to be essentially post-mitotic such as the brain (Weissman, 2000). Since many differentiated cell types are inherently short-lived, or are lost through general wear and tear, and injury, stem cells must function throughout the lifetime of the organism in order to maintain tissue homeostasis, and avoid the onset of tissue-specific atrophy and aplasia. Nonetheless, evidence indicating that aged tissues have a diminished capacity to maintain homeostasis, or return to homeostatic state after exposure to stress, or injury, has implicated stem cell decline in the aging process.

Hematopoietic stem cells (HSC) are the most primitive cells of the blood lineage and are capable, at the single cell level, of giving rise to the entire repertoire of mature blood cells. Lifelong blood cell production is achieved by the capacity of stem cells to both differentiate, and self-renew, properties which establish the primary level of homeostatic control within the hematopoietic system. However, since hematopoietic development is hierarchically structured, homeostatic control of blood cell production is also mediated at the level of downstream multipotent, oligopotent and lineage-restricted progenitor cells. We therefore suggest that a comprehensive understanding of the cellular and molecular etiology of age-associated hematological conditions involving homeostatic imbalance may best be informed by approaching the problem from the context of hematopoietic stem and progenitor cell biology.

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Expansion of LT-HSC with age is cell autonomous

Numerous studies have demonstrated that all hematopoietic stem cell activity in many strains of mice is contained within a minor bone marrow (BM) population with a cell surface phenotype of c-kit positive, lineage negative and Sca-1 positive fraction (KLS cells) (Ikuta and Weissman, 1992; Li and Johnson, 1995; Spangrude et al., 1988). Despite being enriched for stem cell activity however, only a minor subset (~1/30) of KLS cells are in fact stem cells with long-term multi-lineage potential (Bryder et al., 2006). We and others have shown that inclusion of additional cell surface markers such as CD34 (Osawa et al., 1996), and flk2 (Adolfsson et al., 2001; Christensen and Weissman, 2001) can be used to significantly further enrich for HSC activity. Staining the BM of young (3 months), mid-aged (12 months), and old (24 months) mice with these markers reveals 3 distinct subpopulations (KLS: flk2+CD34+, flk2-CD34+, and flk2-CD34-) (Fig. 1A-B). Transplantation of limited numbers of each of these subsets from young and old animals demonstrated that the KLSflk2-CD34+ and KLSflk2+CD34+ populations, which comprise the vast majority of the KLS population of young mice, were only capable of transient lympho-myeloid reconstitution regardless of donor age, consistent with these subsets being non-self-renewing multi-potent progenitors (Rossi et al., 2005). In contrast, all long-term multi-lineage reconstituting activity was contained within the minor KLSflk2-CD34- subset of both young and old mice (Rossi et al., 2005). These data established that BM cells with the surface phenotype of KLSflk2-CD34- are the only cells within the KLS fraction possessing long-term multi-lineage reconstituting hematopoietic stem cell ability (henceforth LT-HSC), and that this stem cell surface phenotype is maintained into old age.

Several studies using different isolation schemes have reported that stem cell BM frequency increases with advancing age (Morrison et al., 1996; Sudo et al., 2000). Indeed, quantification of the BM frequency of KLSflk2-CD34- LT-HSC confirmed that these cells were very significantly increased in frequency with advanced age (Fig. 1B-C) (Rossi et al., 2005). Similar age-dependent increases in stem cell frequency with age were also observed when we used side population (SP) activity (Goodell et al., 1996) (Fig. 1D), or cell surface expression of Slamf1 (Kiel et al., 2005) as criteria for isolating HSC (Fig. 1E) (Rossi et al., 2005). These data demonstrate that LT-HSC identified by multiple strategies are significantly increased in frequency with advancing age. These results highlight the importance of utilizing rigorous stem cell isolation strategies for quantitative evaluation of stem cell function in the context of aging, since assaying stem cell activity based on whole bone marrow, or even KLS cells, as is common in the literature, is extremely problematic due to the enormous changes in stem cell and multi-potent progenitor cell frequencies with age (Fig 1B) (Rossi et al., 2005). The use of KLS or whole bone marrow cells to quantitatively assay the lineage potential of stem cells in the context of aging is further confounded by the fact that the multi-potent progenitor subsets contained within the KLS fraction possess substantial long-term lymphoid, yet only transient myeloid reconstituting potential (Adolfsson et al., 2001; Bhattacharya et al., 2006).

The expansion of the LT-HSC pool with age in the steady-state could be an intrinsic property of these cells, a result of changes in the BM microenvironment with aging, or a combination of both. By assaying the ability of either young or old stem cells to self-renew in young primary transplant recipients we were able to show that LT-HSC from old mice had a greater capacity to give rise to phenocopies of themselves than stem cells from young mice (Rossi et al., 2005). These data demonstrated that the age-dependent steady-state expansion of LT-HSC is a transplantable, cell autonomous property of LT-HSC aging, and thus suggests that stem cell aging is associated with increased self-renewal potential.

Lineage skewing with age is a cell autonomous property of stem cell aging

We and others have evaluated the impact of aging on the lineage potential of stem cells by transplanting limited numbers of purified LT-HSC from young and old mice into young congenic recipients, followed by analysis of donor cell contribution to B-, T-, and myeloid cell lineages at multiple time points post-transplant (Rossi et al., 2005; Sudo et al., 2000). These studies demonstrated that total donor reconstitution was consistently diminished in mice transplanted with old HSC at all time points measured (Fig. 2A). Analysis of the lineage distribution of donor-derived cells demonstrated that LT-HSC from old mice were intrinsically impaired in their ability to give rise to peripheral B-lymphocytes, while at the same time exhibiting an increased propensity to generating Cd11b+ (Mac1) myeloid cells (Fig. 2A) (Rossi et al., 2005).

We also tested whether the aged BM microenvironment could negatively modulate B-cell output, by transplanting purified LT-HSC from young donors into young and old recipients and found that while the old BM microenvironment adversely impacted B-cell production short-term, long-term analyses revealed that the ability of young stem cells to generate mature B-cells was unaffected by the age of the BM microenvironment (Rossi et al., 2005). Taken together, these results suggest that the deficiency in B-lymphopoiesis that accompanies steady state aging is a cell autonomous property of LT-HSC aging that is, to a large degree, independent of the aging BM microenvironment.

Although LT-HSC are ultimately responsible for the production of all mature blood cells, successful production of mature blood cells is contingent upon differentiation through a succession of progenitor cells with increasingly more restricted lineage potential. Analysis of the steady state frequencies oligo-potent myeloid progenitors (Akashi et al., 2000) in young and aged mice revealed that the BM frequencies of the common myeloid progenitor (CMP), and megakaryocyte-erythrocyte progenitor (MEP) were unaffected by age, while old animals exhibited a small but significant increase in granulocyte-macrophage progenitors (GMP) (Fig. 2B) (Rossi et al., 2005). In contrast, analysis of purified common lymphoid progenitors (Kondo et al., 1997) expressing flk2+ (CLP^{flk2+}) (H. Karsunky and I.L.W., submitted) revealed that this progenitor was significantly decreased in old mice (Fig. 2B) (Rossi et al., 2005). Importantly, transplantation of young and old LT-HSC into young recipients revealed that the diminished capacity to give rise to lymphoid progenitors, and the robust capacity to give rise to early myeloid progenitors were transplantable, cell autonomous properties of hematopoietic stem cell aging (Rossi et al., 2005).

Systematic changes in the transcriptome of HSC contribute to the onset of age-associated hematopoietic decline

The discovery that many of the functional attributes of LT-HSC aging were cell autonomous properties prompted us to examine the molecular mechanisms underlying LT-HSC aging. To this end, we performed whole genome micro-array analysis on purified LT-HSC isolated from young and old mice, which revealed that 907 genes (out of ~ 34,000 genes screened) were age-regulated to a high degree of statistical confidence (Rossi et al., 2005). To our knowledge this data set represents the first examination of the genome wide transcriptional changes associated with stem cell aging, and thus represents a valuable resource for the exploration of the impact of aging on the transcriptome of stem cells and LT-HSC biology in general (Rossi et al., 2005). This data freely accessible to the scientific community at <http://genome-www5.stanford.edu/>.

Interestingly, the data from our micro-arrays indicated that regardless of age, LT-HSC express a great diversity of transcripts including genes previously believed to be restricted to more mature and lineage committed cell types. These observations had in fact been foreshadowed

by previous micro-array studies (Akashi et al., 2003;Phillips et al., 2000), and studies using single-cell PCR strategies (Hu et al., 1997;Miyamoto et al., 2002) suggesting that transcription of lineage-associated transcripts in stem cells is antecedent to lineage commitment, and may be required to prime stem cells for differentiation. It was therefore striking that many such lineage-associated genes were differentially expressed in young and old HSC (Rossi et al., 2005). This included a large number of genes involved in specifying lymphoid fate and function, the overwhelming majority of which were down-regulated with age (Table 1) (Rossi et al., 2005). By contrast, a large number of genes involved in mediating myeloid specification and function were found to be upregulated with age (Table 1) (Rossi et al., 2005). These data argue that the age-dependent downregulation of genes mediating lymphoid specification and function, and upregulation of genes mediating myeloid specification function combine in a concerted program to skew the lineage potential of HSC from lymphopoiesis towards myelopoiesis with old age, as was demonstrated by our functional studies.

We also observed that HSC aging was concomitant with overexpression of a large number of genes known to be involved in myeloid leukomogenesis, including prominent proto-oncogenes such as Fgfr1, Aml1, Pml, and Eto (Table 1) (Rossi et al., 2005). Such genes are typically rendered oncogenic through specific cytogenetic rearrangements that juxtapose disparate loci resulting in the production of oncogenic fusion proteins. Since advanced aging is associated with markedly elevated incidences of myeloid leukemias involving these genes, we suggest that the coordinate overexpression of such leukemic proto-oncogenes with age in stem cells might increase their susceptibility to such cytogenetic rearrangements, and thereby predispose aged stem cells to oncogenic events. The mechanism driving such events may be akin to those resulting in immunoglobulin class switch recombination that are transcription-dependent (Chaudhuri and Alt, 2004), and could involve coordinated transcriptional control between interchromosomal loci (Ling et al., 2006;Spilianakis et al., 2005).

Cumulatively, our data on the functional and molecular characterization of LT-HSC aging supports a model in which age-dependent alterations in gene expression at the stem cell level significantly impact differentiation and lineage potential, and thereby factor into major pathophysiological characteristics of the aged hematopoietic system (Fig. 3). In this model, the downregulation of genes mediating lymphoid fate with age, combined with the diminished potential of aged HSC to give rise to lymphoid progenitors, and mature lymphocytes, contribute to the decline of the adaptive immune system with age. By contrast, increased HSC self-renewal, the upregulation of genes specifying myeloid fate, the increased propensity of aged HSC to commit towards myeloid lineages, and the age-dependent upregulation of a core set of proto-oncogenes translocated in the majority of human myeloid leukemias suggests a novel mechanism leading to the increased incidence of these diseases in the elderly.

The future of hematopoietic stem cell aging

In contrast to the growing body of evidence detailing the impact of aging on the functional capacity of HSC, much less is known about the processes driving these changes. The recognition that a number of different human progeria syndromes are attributable to mutations in genes involved in diverse DNA repair pathways, has strongly implicated DNA damage as a mechanism contributing to age-associated decline (Martin, 2005). The prevalence of specific cytogenetic abnormalities in multiple age-associated myeloproliferative disorders is illustrative of the fact that genetic changes arising from genomic instability underwrite diverse pathophysiological aspects of the aging hematopoietic system. However, the contribution of age-accumulated genomic damage to other aspects of HSC aging, such as the lymphoid/myeloid lineage skewing, is much less clear. In fact the systematic changes in expression of lineage specification genes that appears to underwrite lineage bias is more suggestive of a mechanism involving coordinated regulatory control, rather than the stochastic outcomes that

would be predicted if DNA damage was driving these processes. Indeed, evidence that a coordinated global regulatory mechanism might be contributing to certain aspects of LT-HSC aging is revealed by the cadre of age-regulated genes that are known to be involved in higher order chromosome dynamics, chromatin remodeling, and epigenetic regulation of gene expression (Table 1) (Rossi et al., 2005). It therefore appears likely that genetic and epigenetic mechanisms contribute to distinct aspects of stem cell aging and the aging of the hematopoietic system, the elucidation of which will surely inform strategies aimed at ameliorating age-associated hematopoietic decline.

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Fig 1.

Cell autonomous expansion of LT-HSC with age

A) Schematic representation of the primitive hematopoietic compartment showing the lineage relationship and cell surface phenotype of long-term reconstituting hematopoietic stem cells (LT-HSC), and multi-potent progenitor cells. B) Representative FACS profiles of young, mid-aged, and old BM cells showing LT-HSC (red boxes) and multi-potent progenitor cell subsets (blue and green boxes). Cells were gated (boxes) through FSC-A vs. lineage, c-kit vs. Sca1, and flk2 vs. CD34 as shown. C) BM frequency of LT-HSC from young (n=5), middle-aged (n=5) and old mice (n=5) presented as a percent of the young animals. D) BM frequency of $KLSflk2-CD34-$ side population cells young (n=5), and old mice (n=5) presented as a percent of the young animals. E) BM frequency of $KLSCD34-Slamf1+$ cells young (n=5), and old mice (n=5) presented as a percent of the young animals. Statistically significant ($P < 0.05$ by student's t-test) differences are indicated with asterisks. Double asterisks in B indicate statistical significance ($P < 0.05$) to both of the other age groups.

Fig 2.**Lymphoid/myeloid lineage skewing of LT-HSC with age**

A) Representative flow cytometric profiles showing peripheral blood analysis of young recipient mice competitively transplanted with 50 LT-HSC (CD45.2) against 2×10^5 competitor BM cells (CD45.1) from young (top panels) or old (bottom panels) donors showing total donor cell contribution (green contours, left panels), and contribution to B220+ B-cells (red contours, right panels) and Mac1+ myeloid cells (blue contours, right panels) 20 weeks post-transplant. The B220⁻, Mac1⁻ cells (purple contours) are TCRβ⁺ T-cells (not shown). Cells were pre-gated through FSC-A vs SSC, and Ter119 vs PI to exclude erythroid cells and dead cells. B) Representative flow cytometric profiles of committed myeloid progenitor cells from the BM of young and old mice showing common myeloid progenitors (CMP, blue contours), granulocyte-macrophage progenitors (GMP, purple contours) and megakaryocyte-erythroid progenitors (MEP, red contours). Cells were pre-gated through FSC-A vs. lineage (not shown). Representative flow cytometric profiles of flk2⁺ common lymphoid progenitors (CLP^{flk2+}, red contours) from the BM of young and old mice. Cells were pre-gated through FSC-A vs. lineage (not shown).

Fig 3.

Model of hematopoietic stem cell aging

A model for how the cellular and molecular changes accompanying hematopoietic stem cell aging contribute to clinically significant pathophysiological conditions of the aged hematopoietic system.

Table 1

Selected age-regulated genes of different functional categories. Down-regulated genes indicated with ↓, and up-regulated genes with ↑.

Function/Gene Title	Gene	Fold Change
Lymphoid Lineage Specification/Function		
Interleukin 7 receptor	IL7r	↓ 13.7
FMS-like tyrosine kinase 3	Flt3	↓ 3.0
c-srk tyrosine kinase	Csk	↓ 1.6
Phosphoinositide-3-kinase adaptor protein 1	Pik3ap1/BCAP	↓ 2.3
Tumor necrosis factor receptor superfamily 13c	Tnfrsf13c/Baff	↓ 3.3
B-cell leukemia/lymphoma 11B	Bcl11b	↓ 12.9
SRY-box containing gene 4	Sox4	↓ 2.1
Myeloid Lineage Specification/Function		
Friend leukemia integration site 1	Fli-1	↑ 2.6
CCAAT/enhancer binding protein beta	Cebpb	↑ 1.9
CCAAT/enhancer binding protein delta	Cebpd	↑ 5.1
Oncostatin M receptor	Osmr	↑ 6.9
Homeo box B6	Hoxb6	↑ 2.7
Promyelocytic leukemia	Pml	↑ 5.1
Runt related transcription factor 1	AML1/Runx1	↑ 1.6
CBFA2T1 identified gene homolog	ETO/Cbfa2t1h	↑ 1.8
Fibroblast growth factor receptor 1	Fgfr1	↑ 2.0
Myeloid Leukemia		
Promyelocytic leukemia	Pml	↑ 5.1
Runt related transcription factor 1	Runx1/AML1	↑ 1.6
CBFA2T1 identified gene homolog	Cbfa2t1h/ETO	↑ 1.8
Fibroblast growth factor receptor 1	Fgfr1	↑ 2.0
Fibroblast growth factor receptor 3	Fgfr3	↑ 2.0
FGFR1 Oncogene partner 2	Fgfr1op2	↑ 1.7
Rho guanine nucleotide exchange factor	Arhgef12	↑ 2.0
NCK interacting protein with SH# domain	Nckipsd	↑ 2.7
Epigenetic Regulation/Chromatin Remodeling		
Enhancer of zeste homolog 2	Ezh2	↓ 1.7
High mobility group AT-hook 1	Hmga1	↓ 3.5
Special AT-rich sequence binding protein 1	Sat1b	↓ 2.7
SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 2	Smarca2/Brm	↑ 2.6