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REVIEW

Hematopoietic stem cell aging and self-renewal

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Abstract A functional decline of the immune system occurs during organismal aging that is attributable, in large part, to changes in the hematopoietic stem cell (HSC) compartment. In the mouse, several hallmark age-dependent changes in the HSC compartment have been identified, including an increase in HSC numbers, a decrease in homing efficiency, and a myeloid skewing of differentiation potential. Whether these changes are caused by gradual intrinsic changes within individual HSCs or by changes in the cellular composition of the HSC compartment remains unclear. However, of note, many of the aging properties of HSCs are highly dependent on their genetic background. In particular, the widely used C57Bl/6 strain appears to have unique HSC aging characteristics compared with those of other mouse strains. These differences can be exploited by using recombinant inbred strains to further our understanding of the genetic basis for HSC aging. The mechanism(s) responsible for HSC aging have only begun to be elucidated. Recent studies have reported co-ordinated variation in gene expression of HSCs with age, possibly as a result of epigenetic changes. In addition, an accumulation of DNA damage, in concert with an increase in intracellular reactive oxygen species, has been associated with aged HSCs. Nevertheless, whether age-related changes in HSCs are programmed to occur in a certain predictable fashion, or whether they are simply an accumulation of random changes over time remains unclear. Further, whether the genetic dysregulation observed in old HSCs is a cause or an effect of cellular aging is unknown.

Keywords Hematopoietic stem cell · Aging (organismal/cellular) · Self-renewal · Genetical genomics · ROS · C57Bl/6 · DBA/2

Introduction

Organismal aging is a complex phenomenon, the causes of which are not well understood. An important aspect of this process is thought to be cellular aging, which can be viewed as a gradual decrease in cell potential over time and particularly over many cellular divisions. This cellular aging can manifest itself as an increased likelihood of apoptosis, a decreased ability to divide (leading to cellular senescence), or any other factor that results in a decline of the normal function(s) of a given cell. In the hematopoietic system, a functional decline of the immune system is known to occur during organismal aging (Linton and Dorshkind 2004; Miller 1996), and a correlation between lifespan and the cycling activity of primitive hematopoietic cells has been described (de Haan et al. 1997). Most hematopoietic cells have a high turnover and need to be continuously replenished, ultimately from a relatively small compartment of cells in the bone marrow (BM) known as hematopoietic stem cells (HSCs). The constant replicative demand placed

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on this rare HSC population provides an ideal model to study aging of a highly replicating tissue type.

Elucidation of the mechanisms of hematopoietic aging could conceivably lead to strategies to prevent or even reverse the decline in immune function with age. Further, manipulation of these processes might allow the ex vivo expansion of the hematopoietic cells without exhaustion. In addition, since it is thought that many of the same mechanisms that contribute to cellular aging also act as suppressors of neoplastic growth (Campisi 2003, 2005), a better understanding of hematopoietic aging may provide novel avenues to treat leukemias by enhancing or re-introducing intrinsic aging pathways.

This review focuses on the mouse as a model system for aging of the mammalian hematopoietic system. First, the relationship between HSC aging and self-renewal will be discussed. Second, the current knowledge of HSC aging will be described, with a focus on the C57Bl/6 mouse strain, which is the best studied mouse strain in this regard. Third, the differences in hematopoietic aging between C57Bl/6 and other strains will be described, with a focus on how these differences can be exploited to further our understanding of the genetic basis for HSC aging. Finally, some of the potential mechanisms of HSC aging will be explored.

HSCs, HSC aging, and HSC self-renewal

The term "stem cell" is a conceptual expression that encompasses an understanding of a biological phenomenon that is difficult to absolutely or definitively identify. However, it is possible to test for cells that have characteristics in line with this conceptual framework by the use of specific stem cell assays that project an experimentally definable identity onto the stem cell concept. Stem cell assays are retrospective measurements that generally rely on the functional ability of primitive cells to generate multiple types of progeny, in addition to their ability to "self-renew", that is, produce daughter stem cells. In the case of HSCs, the extended output of differentiated cells is generally assumed to be a useful indicator of self-renewal, based on the assumption that cells without self-renewal ability (i.e., non-HSCs) will exhaust their ability to produce mature progeny prior to the termination of the assay. This is one of the basic premises behind long-term in vitro assays, such as the cobblestone-area-forming cell assay (CAFC; Ploemacher et al. 1989; Breems et al. 1994) or long-term culture-initiating cell assay (LTC-IC; Dexter et al. 1977; Sutherland et al. 1989), and most in vivo long-term repopulation assays (Harrison 1980; Szilvassy et al. 1990). However, since HSCs are defined by their "read-out" in a particular assay, the most rigorous and relevant definition of self-renewal is to test daughter cells for HSC activity by using the same assay as that applied to the starting population.

Whereas the ultimate purpose of HSC assays is to test for the functional ability of a cell to perform during normal hematopoiesis, it is important to realize that each assay places additional demands on the cell. Depending on the nature of the assay, any one of a variety of cell properties might be deficient, thereby preventing a cell from being identified as an HSC. In general, a negative response in an HSC assay does not distinguish whether the cell has differentiated, died, or senesced, or further (specifically in *in vivo* assays), if it had become immunogenic, was not successful in migrating into the BM or was been unable to home and respond to an appropriate niche. However, since a hematopoietic stem cell is experimentally defined by its assay, any cell that does not "read-out" in that assay, regardless of the reason, cannot legitimately be classified as a HSC. Hence, experimental criteria that permit the proper interpretation of the term "hematopoietic stem cell" in any given study must be clearly defined.

HSC self-renewal is defined at the population level as the maintenance or expansion of stem cell numbers following cellular divisions. At the single cell level, this definition is less clear. For example, in studies where the self-renewal capacity of individual HSCs were demonstrated (Ema et al. 2005; Dykstra et al. 2007), the measurements of self-renewal were logically restricted to the definition (assay) used to define the presence or absence of an HSC. Because any HSC assay is a relatively crude functional measurement, identification of HSC self-renewal in this manner does not preclude the possibility that the daughter HSCs are actually slightly different from the parent HSC. In reality, whether a daughter HSC shares exactly the same properties as its parent is fundamentally impossible to determine, since the parent no longer exists in its original form as soon as cell division occurs. In addition, it is not feasible to measure every relevant parameter to determine whether two cells are functionally identical, and so our best attempts to define functional equivalency are merely crude approximations of molecular events being implicated but not yet clarified.

As described in this review, the HSC compartment is well known to undergo considerable age-related changes. Whether these changes are intrinsic to the HSCs themselves or whether they occur because of alterations in the composition of cells within the HSC compartment is not yet clear (Fig. 1). If HSC aging is primarily attributable to intrinsic alterations, an interesting paradox is raised, since at the level of a single HSC, cellular aging and true self-renewal (in its most literal sense) are mutually exclusive properties. If age-related changes derive from relative alterations of specific subtypes within the HSC compartment, this adds a new layer of complexity to the currently accepted paradigms of HSC biology and offers new oppor-

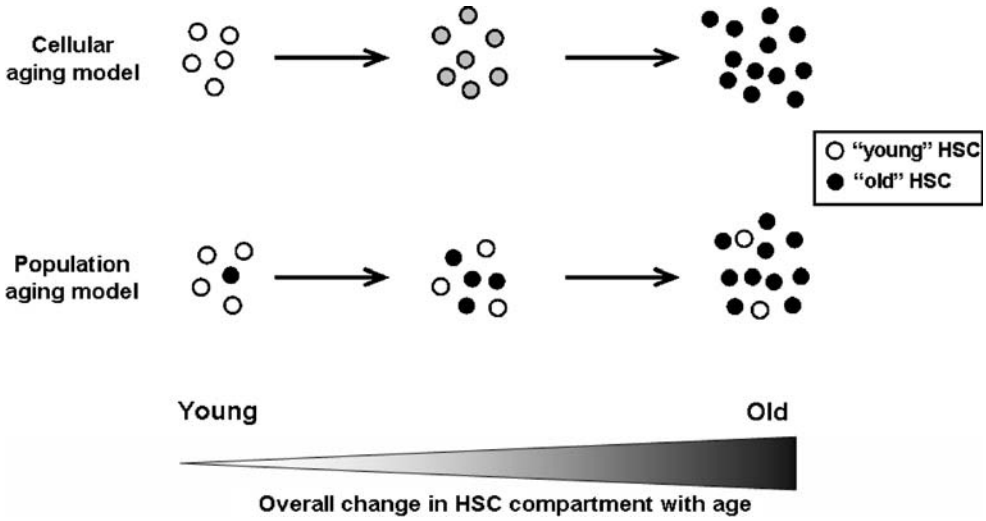

Fig. 1 Age-related changes within the HSC compartment may be dependent or independent of cellular aging of HSCs. One explanation is that gradual changes (whether random or directed) take place within all HSCs with age (*top*), and is therefore inconsistent with the idea of "true" HSC self-renewal. An alternate explanation is that the hematopoietic stem cell pool contains a heterogeneous mixture of HSCs, each with intrinsically determined, stable characteristics. With age, an overall change in the function of the HSC compartment occurs as a result of a relative decrease in some HSC subsets and a relative accumulation of other HSC subsets (*bottom*). This could be attributable to either intrinsic differences in self-renewal likelihoods, or a response to the changing environment of the aging mouse, which may introduce selective pressures that alter the self-renewal likelihood of different HSC subsets. Since careful studies of individual HSCs have not yet been completed in this context, the means by which the HSC compartment changes with age remains unresolved

tunities for the understanding and potential modification of the hematopoietic aging process.

Hematopoietic aging may be in part attributable to environmental (non-intrinsic) changes but is primarily caused by intrinsic alterations in the HSC compartment

Since all hematopoietic cells derive from the HSC compartment, age-related changes of hematopoiesis must be a result of some combination of intrinsic alterations in the HSCs themselves and/or alterations in the hematopoietic environment that affect the behavior of the HSCs or their downstream progeny. Examples of intrinsic alterations might include modifications in DNA sequence, epigenetic alterations, and/or oxidative damage leading to changes in the levels or activity of cellular proteins, such as cell-surface receptors, signalling intermediates, or transcription factors. Examples of environmental alterations might include changes in the cell-surface protein repertoire of, or factors excreted by, stromal cells, osteoblasts, or other components of the local microenvironment (commonly referred to as the HSC niche). In addition, a systemic increase or decrease in hematopoietic cytokine levels or other factors important for hematopoietic cell differentiation might also contribute to the changing characteristics of the hematopoietic system with aging.

A number of studies have shown that an aged hematopoietic environment can have an effect on hematopoiesis. Subcutaneous implantation of femoral bones from young and old mice resulted in a decreased repopulation of host hematopoietic progenitors in the old femur compared with the young femur (Hotta et al. 1980), suggesting that the environment within the old implanted bone is deficient in its ability to attract or maintain primitive hematopoietic cells compared with that of the young implanted bone. This may be in part attributable to age-related changes of stromal cells, which are an important component of the supportive HSC niche within the BM. Supporting this concept, an age-related change in stromal cells was observed in vitro using a long-term BM culture system, where the hematopoietic progenitor output of young BM was shown to be decreased when cultured on stroma from old versus young mice (Mauch et al. 1982). That the environment could affect not only hematopoietic progenitors, but also HSCs themselves was shown elegantly in vivo by Liang et al. (2005) who demonstrated a reduced ability of both hematopoietic progenitors and HSCs to home to the BM or spleen upon transplantation into old versus young recipients. In addition, a myeloid-lineage-skewing characteristic of aged hematopoiesis was observed in the initial months after young HSCs were transplanted into old compared with young recipients (Rossi et al. 2005). That alterations in the BM environment can be causative for abnormal hematopoiesis was recently demonstrated by Walkley et al. (2007), who showed that normal BM cells injected into RAR-gamma knockout mice were not able to cure the myeloproliferative disorder normally observed in this mouse,

whereas the transplantation of RAR-gamma knockout cells into wild-type recipients resulted in normal hematopoiesis.

However, an increasing body of evidence suggests that the majority of the age-related changes observed in the hematopoietic system are caused by intrinsic alterations in the HSCs themselves. Before undertaking a survey of this evidence, it is important to note that most mouse aging studies in recent years have been performed on the C57Bl/6 background, which, as will be discussed later, is somewhat anomalous with respect to its HSC aging characteristics when compared with other mouse strains. Therefore, literature describing HSC aging in the C57Bl/6 strain will first be reviewed separately and then compared with the aging phenotypes seen in other strains.

HSC numbers in C57Bl/6 mice increase with age, concurrently with an increase in cycling activity and a decrease in engraftment efficiency

A quarter of a century ago, BM cells from old C57Bl/6 donors were shown to outperform an equivalent number of BM cells from young donors, when assessed for long-term erythropoiesis in a competitive transplant setting (Harrison 1983). Similar experiments performed more recently have reiterated these findings, with old BM demonstrating a two- to three-fold increase in competitive repopulating ability (measured by levels of white blood cell chimerism) compared with young BM (Morrison et al. 1996; Sudo et al. 2000). However, of note, these types of measurements indicate only the total repopulating ability of a bulk population of BM and cannot distinguish whether, during aging, a change occurs in the number of HSCs, the competitive repopulation ability (quality) per HSC, or some combination thereof.

This unresolved question was first dealt with by the use of a statistical method to estimate the numbers of HSCs based on competitive repopulation results; the frequency of HSCs was suggested to increase two-fold in old (>2 years) versus young (2–3 months) donors (Harrison et al. 1989). More recently, with a limiting dilution approach, the frequency of HSCs detectable in old BM was also found to be two-fold higher (Liang et al. 2005; Sudo et al. 2000), whereas the average repopulation ability per HSC remained constant (Liang et al. 2005; Morrison et al. 1996). Similar experiments performed using the CAFC assay, an in vitro measurement for HSCs, revealed an even greater increase of the most primitive hematopoietic cells with age: approximately four–fold (de Haan and Van Zant 1999a).

Using flow cytometric techniques, it was also shown that the phenotypically defined HSC-enriched population defined by the cell surface markers c-Kit^{+}Thy1.1^{lo}Sca-1^{+}Lin^{-} (KTSL) were increased seven-fold in old versus young C57Bl/6 mice (Morrison et al. 1996). Similar increases in frequency with age, ranging from six- to 17-fold, have been reported when comparing other phenotypically defined populations highly enriched for HSCs (Rossi et al. 2005; Sudo et al. 2000; Chambers et al. 2007; Pearce et al. 2004; Rossi et al. 2007a). Of note, upon transplantation into young irradiated recipients, HSCs from old mice have a greater capacity to give rise to phenocopies of themselves than HSCs from young mice, suggesting that the increased generation of these cells is an intrinsic characteristic of old HSCs, rather than the product of an old hematopoietic environment (Rossi et al. 2005). At the same time, however, the frequency of phenotypically defined HSCs able to repopulate recipients upon transplantation *in vivo* was observed to decrease in old donor mice by four- to seven-fold (Rossi et al. 2005; Morrison et al. 1996). Collectively, these age-related changes are consistent with the two-fold increase in the overall repopulating ability of BM from old versus young C57Bl/6 mice.

A common interpretation of these results is that the frequency of HSCs within these phenotypically defined HSC-enriched populations is actually constant throughout aging, but there is a concurrent intrinsic age-related decrease in the ability of aged HSCs to successfully engraft in an in vivo transplantation setting (Morrison et al. 1996). This decrease, estimated to be approximately four- to six-fold for HSCs in old BM (Morrison et al. 1996), could conceivably be attributable to a failure of any of the steps requisite for long-term repopulation, including initial cell survival, homing to the BM, appropriate localization within the BM niche, or to the long-term maintenance of appropriate self-renewal and differentiation decisions. An alternate hypothesis is that there is simply an increase with age of the proportion of contaminating non-HSCs sharing the surface markers of the enriched population (Sudo et al. 2000). In this eventuality, the contaminating cells would probably be primitive progenitors, since most phenotypically purified cells from old C57Bl/6 mice retain their clonogenic ability in stromal and stromal-free cultures (Morrison et al. 1996; Sudo et al. 2000).

Recent papers have shed some light on this issue. Following on their earlier work, which showed that BM cells of the KTSL phenotype can be subfractionated into functionally distinct subtypes based on their expression of the SLAM markers CD150 and CD48 (Kiel et al. 2005), Yilmaz et al. (2006) compared the SLAM profiles of KTSL cells from the BM of young and old C57Bl/6 mice. Within the KTSL population of old BM, the proportion of CD48^{+} cells (which are devoid of long-term repopulating activity) increased from 20% to 60%, translating to a two-fold decrease of the HSC-containing CD48^{-} population (Yilmas et al. 2006). When CD48^{-}KTSL or CD150^{+}CD48^{-}KSL cells were tested for long-term in vivo repopulating ability at limiting dilutions of three to five cells, HSC frequencies of 1 in 4 to 1 in 6 were obtained (Yilmaz et al. 2006). Con-

sidering that the frequency of HSCs within these populations in young mice is approximately 1 in 2 (Kiel et al. 2005; Yilmaz et al. 2006), this result suggests that the maximum possible engraftment defect is two- to three-fold, whereas the remainder of the discrepancy between purified HSC populations from young and old mice is probably attributable to an increase in contaminating progenitor cells with age.

In an effort to directly measure one of the variables involved in long-term engraftment efficiency, Liang et al. (2005) determined the 24-h homing efficiency of functionally defined HSCs upon transplantation of young or old BM. Relative to the number of functionally defined HSCs injected, the frequency of HSC recovery 24 h post-transplant decreased two-fold for HSCs from old compared with young donors (Liang et al. 2005). However, since this is only a short-term measurement of homing efficiency, it does not preclude the possibility that HSCs from old mice may preferentially seed elsewhere prior to colonizing the BM. Nevertheless, these results do demonstrate a distinct functional difference between HSCs from old and young C57Bl/6 mice and suggest that a reduced ability to seed to the BM may be a cause of the decreased engraftment efficiency in HSCs from old mice. A complementary study measuring the ability of hematopoietic progenitors (CAFC day 7) to interact with stromal cells in vitro within 2–4 h after seeding showed a decreased adherence to stroma cells isolated from old versus young mice (Xing et al. 2006). An additional observation that suggests an age-related alteration in the interactions between HSCs and their environment is that aged mice show an increased mobilization of HSCs in response to granulocyte-colony-stimulating factor (Xing et al. 2006).

A phenomenon that might be connected to the decreased engraftment efficiency is the reduction in HSC engrafting potential during the progression of HSCs through the S/G2/M phases of the cell cycle (Fleming et al. 1993; Habibian et al. 1998; Bowie et al. 2006). In young mice, the vast majority of phenotypically defined HSCs are quiescent (Cheshier et al. 1999), but with age, a greater proportion are cycling (Morrison et al. 1996). Interestingly, the shift to greater cycling activity does not seem to be gradual with age, since phenotypically defined HSC populations in 18-month-old mice have similar cycling characteristics to that of young C57Bl/6 mice, whereas the same cell populations in 22- to 24-month-old mice have a three- to four-fold increase in the percentage of cells in the S/G2/M phases of the cell cycle (Morrison et al. 1996; Sudo et al. 2000; Yilmaz et al. 2006). However, this is not the case for all phenotypically defined HSC populations, since the proportion of SP/KSL cells in the S/G2/M phases does not change between 2 and 23 months of age (Chambers et al. 2007). This suggests that the increase in cycling activity in old mice might be exclusive to the non-SP HSCs, which may represent up to half of the HSC pool, at least in young mice (Morita et al. 2006).

A summary of the changes in the HSC compartment reported to occur with age in the C57Bl/6 mouse is presented in Table 1.

Myeloid skewing of differentiation potential with age

The lymphoid differentiation potential of old BM has long been realized to be lower than that of young BM (reviewed in Linton and Dorshkind 2004). That this is an intrinsic property of aged BM, rather than a product of an aged hematopoietic environment, has been shown by removing the cells from their endogenous environment and transplanting them into young mice. For example, when old or young BM was transplanted into young irradiated recipients, myeloid progenitors (measured as day 9 colony-

Table 1 Summary of changes in the HSC compartment with age in C57Bl/6 mice

Magnitude of change	Characteristic	Reference(s)
Two-fold increase	Overall repopulating ability in vivo	Harrison 1983; Morrison et al. 1996; Sudo et al. 2000
Seven- to 16-fold increase	HSC number (cell surface phenotype)	Rossi et al. 2005; Morrison et al. 1996; Sudo et al. 2000; Chambers et al. 2007; Pearce et al. 2004; Rossi et al. 2007a
Four-fold increase	HSC number (in vitro CAFC assay)	de Haan and Van Zant 1999a
Two-fold increase	HSC number (in vivo, limiting dilution)	Liang et al. 2005; Sudo et al. 2000
Two-fold decrease	24-h homing efficiency in vivo	Liang et al. 2005
Two- to seven-fold decrease	In vivo engraftment efficiency of phenotypically identified HSCs	Rossi et al. 2005; Morrison et al. 1996; Yilmaz et al. 2006
Three- to four-fold increase	Frequency of phenotypically identified HSCs transiting the cell cycle	Morrison et al. 1996; Sudo et al. 2000; Yilmaz et al. 2006
Decrease	Lymphoid differentiation potential	Liang et al. 2005; Rossi et al. 2005; Sudo et al. 2000

forming units-spleen) were found to be unchanged with age, but the ability to generate T-cells (measured by thymic regeneration) was reduced (Tyan 1977). A similar phenomenon was also observed with respect to reduced B-cell output, and the resultant myeloid skewing was again shown to be a stem cell intrinsic phenomenon that was independent of recipient age (Kim et al. 2003). These observations were extended using a limiting dilution approach in which individual competitive repopulating units, thought to correspond to individual HSCs, were shown to have a myeloid skewed output pattern in young recipients of old BM, but not in young or old recipients of young BM (Liang et al. 2005). Similarly, Rossi et al. (2005) demonstrated that the transplantation of a small number of CD34-flk2-KSL cells from old BM resulted in slightly higher myeloid cell output and significantly lower B-cell output, both short-term and long-term (4 and 26 weeks post-transplant). When phenotypically defined lineage-restricted progenitor populations were compared in these transplanted recipients, the number of regenerated myeloid progenitors (CMP, GMP, MEP) was equivalent, whereas there were two-fold less common lymphoid progenitors (CLP) regenerated in the recipients of old HSCs compared with transplants of young HSCs (Rossi et al. 2005). Thus, the lineage skewing phenotype might be traced back to differences in the production of the earliest lineage-restricted progenitors, suggesting that the differences are probably intrinsic to the stem cells themselves.

Further evidence that these differences are intrinsic to HSCs has been provided by the experiments of Sudo et al. (2000) who demonstrated that the tendency for defective lymphoid production is a property that can be inherited by HSC progeny generated in vivo. In mice transplanted with 10 old (18 months) CD34-KSL cells, strong myeloid skewing was seen in most recipients. An additional round of transplantations was then performed in which regenerated BM from three of these mice were transplanted into groups of secondary recipients. In one of the three groups, all the secondary recipients showed balanced outputs of lymphoid and myeloid cells, whereas in the other two groups, all recipients showed reduced lymphoid progeny (Sudo et al. 2000). Therefore, this lineage skewing appears to be attributable to a subset of HSCs (termed “defective” HSCs by Sudo et al. 2000) that accumulate with age, rather than all HSCs acquiring this aged characteristic (see Fig. 1). That a subset of HSCs might have an intrinsically determined, heritable, myeloid-biased output has been suggested by others (Dykstra et al. 2007; Muller-Sieburg et al. 2002). However, these studies have not included HSCs from old mice in their analysis, and so at this point, their relationship to the “defective” HSCs described by Sudo et al. (2000) can only be speculated. If these cells are equivalent, the mechanism of myeloid skewing in old mice might be related to a blunted response to the lymphoid growth factor interleukin-7 (Muller-Sieburg et al. 2004).

C57Bl/6 mouse strain has unique HSC aging characteristics not representative of other mouse strains

It is important to realize that many of the changes described to occur in aged HSCs are unique to the C57B1/6 genetic background and are not observed when similar experiments are performed on HSCs from other mouse strains. Although aging studies in other strains have not been completed in as much depth as those in C57Bl/6 mice, several differences have become clear, providing strong evidence for a distinct genetic component to aging. These differences illustrate the caution that must be taken when attempting to apply knowledge gained from genetically homogeneous experimental models to genetically heterogeneous populations such as humans. Nevertheless, the variation seen between inbred mouse strains offers a powerful tool to study the genetics of aging in HSCs.

As mentioned above, various intrinsic alterations have been described to occur in HSCs with aging in C57Bl/6 mice, including reduced lymphoid cell production, increased HSC cycling, and an engraftment defect. However, on this genetic background, these alternations do not appear to have particularly drastic or detrimental effects on the overall function of the hematopoietic system; indeed, because of an increase in the absolute number of HSCs, overall function is increased when compared with young BM. Moreover, even when HSCs from C57Bl/6 mice were forced to perform well beyond a normal lifespan in the context of serial transplantation, little functional difference was observed between old and young BM (Chen et al. 2000a; Harrison and Astle 1982; Ogden and Mickliem 1976).

In contrast, a decrease in competitive repopulating ability was observed in old versus young BM from CBA, Balb/c, and DBA/2 strains, and the decrease was even more striking when the cells were subjected to several rounds of serial transplantation (Harrison 1983; Chen et al. 2000b; Kamminga et al. 2005).

A set of particularly informative experiments was performed by Van Zant et al. (1990) who compared the intrinsic in vivo repopulation abilities of HSCs from C57Bl/6 and DBA/2 mice. Mice chimeric for DBA/2 and C57Bl/6 cells were generated by the aggregation of 8-day embryos. The resulting chimeras contained a mixed population of DBA/2 and C57Bl/6 HSCs, and the contributions of erythrocytes and hematopoietic progenitors produced from each HSC type were then tracked with age. Whereas the DBA/2 HSCs initially produced stable levels of erythroid cells, after approximately 1.5 years of age, the DBA/2

erythroid contribution began to decrease relative to the contribution from the C57Bl/6 HSCs. This was a common and reproducible phenomenon, with 22 of 27 allophenic mice showing a dramatic shift of more than 25% (Van Zant et al. 1990). In addition, to compare the kinetics of hematopoietic recovery produced by DBA/2 and C57Bl/6 HSCs in a competitive transplantation setting, radiation chimeras were generated by transplanting allophenic marrow into lethally irradiated F1 recipients. In the initial 2–3 months after transplantation, the regenerated hematopoietic progenitors, identified by their ability to produce colonies in methylcellulose cultures, were primarily of DBA/2 origin. However, the proportion of progenitors of C57Bl/6 origin steadily increased at later times post-transplant. The proportion of erythrocytes derived from DBA/2 or C57Bl/6 HSCs followed a similar, albeit somewhat delayed, pattern of reconstitution (Van Zant et al. 1990). Together with earlier results showing a dramatically reduced rate of cycling progenitors in C57Bl/6 compared with DBA/2 mice (Van Zant et al.1983), this led to the hypothesis that a genetically determined, cell autonomous difference in the proliferation rate of HSCs resulted in an initial competitive advantage of DBA hematopoiesis, although this was at the expense of longevity, since the DBA/2 HSCs appeared to exhaust more rapidly.

The hypothesis that age-related changes in HSCs are highly strain-dependent was strengthened by the observation that the frequency of CAFC d35 cells, an in vitro measure of HSCs, was initially higher in young DBA/2 BM than in C57B1/6 BM. However, the frequency of these cells dramatically decreased after 1 year of age in DBA/2 mice, whereas they continued to increase throughout the lifespan of C57Bl/6 mice (Fig. 2; de Haan and Van Zant 1999a).

Of particular interest with respect to aging, a correlation has been described between organismal lifespan and the extent of cycling in primitive hematopoietic cells (de Haan et al. 1997). This strongly suggests the existence of a genetic determinant impacting both HSCs and overall organismal aging. Through the use of recombinant inbred strains (see below), quantitative trait loci have been identified, highlighting specific gene regions that may contribute to these two related phenotypes (de Haan and Van Zant 1999b; Henckaerts et al. 2004).

It is also important to consider the potential impact of strain-dependent differences when using knockout or overexpression methods to study HSC function. One recent and relevant example is that of p21. When p21-deficient HSCs from 129/SV mice were compared with their wild-type counterparts, their overall numbers and cycling activity were increased. In addition, the p21-deficient HSCs were shown to have an acute sensitivity to hematological stresses as demonstrated by reduced survival upon serial transplantation or repeated 5-FU administration. Together, these observations led to the conclusion that p21 is a crucial

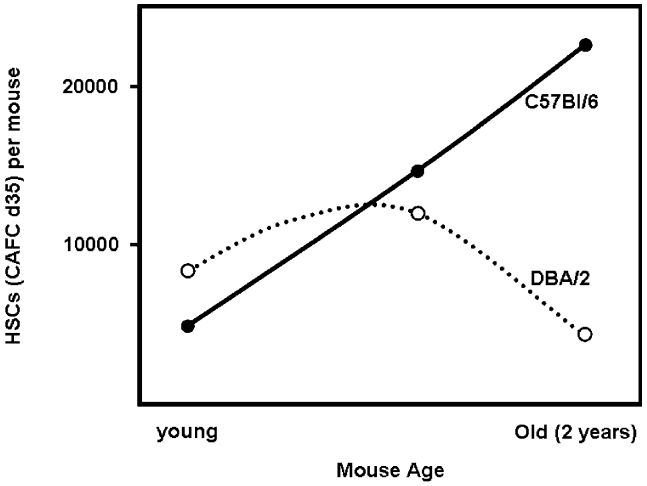

Fig. 2 Age-related changes in HSC number are highly strain-dependent. Measurement of the total number of HSCs per mouse by using the in vitro CAFC assay reveals dramatic differences in HSC numbers with age in C57Bl/6 and DBA/2 mice. Adapted from (de Haan and Van Zant 1999a)

mediator of hematopoietic stem cell cycle initiation, with its deficiency leading to rapid HSC senescence (Cheng et al. 2000). However, when the effects of p21 deficiency were studied in HSCs from C57Bl/6 mice, much more limited effects were seen (van Os et al. 2007), suggesting that alternative mediators of HSC cycling and senescence are active in these two mouse strains.

Exploitation of the unique aging properties of C57Bl/6 HSCs by analysis of recombinant inbred strains

Although the C57Bl/6 strain might not be perfectly representative of "normal" hematopoietic aging in mice, the differences between C57Bl/6 and other strains provides a valuable strategy for investigating the genetic regulation of aging. In particular, the use of recombinant inbreds provides a powerful approach to study the genetic control of HSC aging (for a review, see Snoeck 2005). Recombinant inbred lines are formed by the crossing of C57Bl/6 with other inbred strains, followed by repeated brother-sister mating. In this manner, new inbred lines are produced whose genomes are mosaics of the two parental genomes. Panels of inbred strains have been created in which the genome of each line has been mapped in detail, and such panels represent useful tools for examining the genetic regulation of a host of phenotypes that are different between the parental strains. Multiple genetic loci responsible for differences in the frequency and cycling status of HSCs and early progenitors, and their change with age, have been identified in this manner (Henckaerts et al. 2004; Liang and Van Zant 2003; Geiger et al. 2005). Genetic loci involved in controlling the expression of nearby or distant

genes have also begun to be identified using a "genetical genomics" approach in which global gene expression data is collected for each of the inbred strains, and the transcript abundance of each gene is treated as a unique trait that can then be mapped to a particular genetic region (de Haan et al. 2002; Bystrykh et al. 2005; database available at http://www.genenetwork.org). Combining these approaches has led to the identification of *Lxn* as a gene involved in regulation of HSC pool size (Liang et al. 2007).

HSC senescence or exhaustion is the final result of extensive cellular aging

In addition to the more subtle age-related changes in HSCs discussed earlier in this review, another important aspect of aging should be considered. The exhaustion of HSC self-renewal potential, resulting in terminal differentiation or irreversible senescence, is an inevitable outcome of a large number of cellular divisions. In the absence of stressors, exhaustion of the entire HSC pool does not normally occur during the lifetime of a mouse. However, treatment with cytotoxic drugs or ionizing radiation can induce premature exhaustion of HSC activity (Meng et al. 2003). In addition, serial transplantation, each round of which is estimated to be equivalent to four normal lifespans of hematopoiesis (Harrison et al. 1978), leads to HSC exhaustion after four to five rounds of transplantation (Harrison and Astle 1982). Whereas the relationship between induced replicative senescence and "normal" HSC aging is not well established, studies of this type nevertheless provide valuable insight into the mechanisms controlling the overall replicative lifespan of tissue-specific stem cells.

Mechanisms of cellular aging of HSCs

Although the molecular processes leading to HSC aging have not yet been elucidated, several mechanisms have been identified that may play a role. One well-known mediator of cellular aging is that of telomeres, which are thought to be a "mitotic clock", shortening with successive cellular replications. Whereas the telomere-elongating enzyme telomerase is known to be expressed in HSCs, its expression is limited, and thus, telomere shortening does still occur (for a review, see Zimmerman and Martens in this issue; DOI 10.1007/s441-007-0495-2). Nevertheless, although a telomerase deficiency was shown to limit the replicative lifespan of HSCs in a serial transplantation setting (Allsopp et al. 2003a), overexpression of telomerase did not increase their serial transplantability (Allsopp et al. 2003b), suggesting that telomerase is not the limiting factor that induces HSC exhaustion in serially transplanted mouse HSCs. A recent report has indicated that the telomeres' effects on HSC aging are mediated by the p53 effector $p21^{Cip1Waf1}$, since p21 deletion rescued the negative effects of short telomeres on HSCs without increasing carcinogenesis (Choudhury et al. 2007). Conversely, the deletion of p53 also rescued the negative effects of telomere loss, but with an increase in carcinogenesis (Chin et al. 1999). Recently, Dumble et al. (2007) reported that p53 dosage could impact HSC aging. Lower p53 doses resulted in increased HSC cycling, a loss of primitive quiescent phenotype, and a more rapid exhaustion. Interestingly, the increase in HSCs seen in C57Bl/6 mice with age was also abrogated with high p53 dose.

In contrast to the "directed" aging mechanisms such as telomere shortening, the random accumulation of cellular damage can also contribute to aging. This accumulation of damage is an unavoidable process, since metabolic by-products such as reactive oxygen species (ROS) are damaging to virtually all intracellular components, including lipids, proteins, and mitochondrial and nuclear DNA (for a review, see Ames et al. 1993). Intracellular ROS has been shown, in many species and cell types, to increase with age and is postulated to be an important causal factor of cellular and organismal aging (for a review, see Sohal and Weindruch 1996). Low levels of intracellular ROS have been associated with quiescence of HSCs, consistent with the finding that these cells are predominantly associated with the osteoblastic niche (Moore and Lemischka 2006), which is one of the most hypoxic regions of the BM (Parmar et al. 2007). Protecting HSCs from cellular damage by limiting both cellular replication and exposure to ROS presumably extends their lifespan by limiting DNA mutations and damage to other intracellular molecules.

In addition, accumulating evidence now suggests that the level of intracellular ROS might itself directly affect HSC fate. This concept is supported by the premature HSC exhaustion observed in mice deficient for the oxidative stress mediators *ATM* or *FoxO* (Ito et al. 2004; Tothova et al. 2007). HSCs from $ATM^{-/-}$ mice had increased levels of intracellular ROS, which was shown to activate the p38 MAPK pathway, causing the cells to exit quiescence and begin proliferation (Ito et al. 2006). That intracellular ROS exerts its effects on HSCs via this signalling pathway was confirmed by the observation that HSC exhaustion in *ATM*-deficient mice could be abrogated by long-term exposure to an antioxidant (N-acetyl-L-cysteine, NAC) or SB203580, a pharmacological inhibitor of p38 MAPK (Ito et al. 2006). These findings were recently extended to normal young C57Bl/6 mice in which HSCs purified on the basis of low intracellular ROS levels were reported to have a higher self-renewal potential, whereas those with high ROS levels were more rapidly exhausted upon serial transplantation and had a myeloid skewed differentiation pattern, reminiscent of that

seen upon the transplantation of aged HSCs (Jang and Sharkis 2007). Again, this did not appear to be a permanent alteration in HSC state, since the ROS^{high} HSCs regained normal function when treated with NAC or SB203580 (Jang and Sharkis 2007). A recent study comparing the gene expression profile of purified HSCs from mice of various ages reported an age-dependent upregulation of genes involved in inflammatory and stress responses (Chambers et al. 2007), suggesting that environmental influences on HSCs are altered in old mice. Whether this is attributable to an age-related change in the HSC niche itself or to a movement of aged HSCs to a different microenvironment is not yet known.

Accumulation of DNA mutations can be a cause of HSC aging, as evidenced by the premature hematopoietic exhaustion observed in mice deficient for any of a number of genes involved in the repair or maintenance of genomic DNA (Bender et al. 2002; Nijnik et al. 2007; Rossi et al. 2007b). However, the accumulation of DNA lesions is also relevant in the context of normal aging in wild-type C57Bl/6 mice. By immunostaining for H2AX phosphorylation (an indicator of double-stranded DNA breaks), the extent of DNA damage was shown to be higher and more prevalent in phenotypically defined HSCs from old mice compared with those from young mice (Rossi et al. 2007b). Interestingly, it was also found that less primitive hematopoietic progenitors from old mice had progressively less DNA damage (Rossi et al. 2007b), suggesting that proliferating progenitor cells either have an increased DNA repair response, or that those stem cells with higher DNA damage are less likely to generate hematopoietic progeny. The latter possibility is an intriguing one, as it leads to speculation that the apparent accumulation of HSCs and the decreased engraftment efficiency discussed earlier might be in part attributable to HSCs that have become senescent because of an accumulation of DNA damage. This might be exacerbated by the decreased expression of DNA repair and maintenance genes observed in old HSCs (Chambers et al. 2007). However, whether increased DNA mutations are a cause or an effect of hematopoietic aging remains uncertain.

Epigenetic changes may also play a role in the aging of HSCs. During differentiation, chromosomes are altered via epigenetic mechanisms, resulting in altered gene transcription and the adoption of a particular cell fate. Epigenetic changes have been hypothesized to be the means by which "cellular memory" is maintained throughout the aging process, thus altering the behavior and potential of the daughter cells (Villeponteau 1997; Imai and Kitano 1998). Supporting this concept is the observation that treatment with drugs that interfere with DNA methylation and histone deacetylation (both vital processes for epigenetic regulation) can halt the culture-induced decline of human HSCs and increase the likelihood of self-renewal (Young et al. 2004; Bug et al. 2005). Epigenetic memory is known to be mediated in part by the Polycomb group of proteins (for a review, see Lund and van Lohuizen 2004). The manipulation of Polycomb genes can influence HSC fate, as demonstrated by the examples of Bmi-1 (Iwama et al. 2004) and Ezh2 (Kamminga et al. 2006) among many others (for a review, see Iwama et al. 2005). Further evidence of age-related epigenetic changes in mouse HSCs was provided by a recent global expression analysis of purified mouse HSCs, which revealed an age-related decrease in the expression of genes involved in chromatin maintenance (Chambers et al. 2007). Groups of genes from multiple chromosomal regions were also observed to change with age, and an overall increase in transcriptional activity occurred, including genes normally restricted to specialized hematopoietic cell types (Chambers et al. 2007).

An important question that remains to be answered is the extent to which age-related changes in HSCs are programmed to occur in a certain predictable fashion and to what extent they are simply an accumulation of random changes over time. Further, whether the genetic dysregulation observed in old HSCs is a cause or an effect of cellular aging remains unknown. Certainly, it is possible that cell-to-cell variability in gene expression increases with age, as was observed in a recent study of young and old cardiac cells (Bahar et al. 2006). However, since gene expression analyses of old versus young HSCs (Rossi et al. 2005; Chambers et al. 2007) were performed using pooled populations of HSCs, the majority of cell-to-cell variation attributable to random genetic or epigenetic changes would be offset, and as a result, this data is suggestive of a co-ordinated regulation of expression. In all likelihood, some combination of random and directed changes in both HSCs and the cells of their supportive niches jointly contribute to the scope of functional changes observed in the hematopoietic system during aging. Further study aimed at elucidating the role of each of these factors will improve our understanding of the cellular aging of HSCs and the age-related decline of the hematopoietic system and hopefully increase our knowledge of organismal aging as a whole.

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