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Concise Review: Hematopoietic Stem Cell Aging and the Prospects for Rejuvenation

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ABSTRACT

Because of the continuous increases in lifetime expectancy, the incidence of age-related diseases will, unless counteracted, represent an increasing problem at both the individual and socioeconomic levels. Studies on the processes of blood cell formation have revealed several shortcomings as a consequence of chronological age. They include a reduced ability to mount adaptive immune responses and a blood cell composition skewed toward myeloid cells, with the latter coinciding with a dramatically increased incidence of myelogenous diseases, including cancer. Conversely, the dominant forms of acute leukemia affecting children associate with the lymphoid lineages. A growing body of evidence has suggested that aging of various organs and cellular systems, including the hematopoietic system, associates with a functional demise of tissue-resident stem cell populations. Mechanistically, DNA damage and/or altered transcriptional landscapes appear to be major drivers of the hematopoietic stem cell aging state, with recent data proposing that stem cell aging phenotypes are characterized by at least some degree of reversibility. These findings suggest the possibility of rejuvenating, or at least dampening, stem cell aging phenotypes in the elderly for therapeutic benefit. STEM CELLS TRANSLATIONAL MEDICINE *2015;4:186–194*

INTRODUCTION

The physiological process of aging is accompanied by an overall loss of fitness and a dramatically increased prevalence of many of our most devastating diseases, including dementia, autoimmunity, and cancer. As the lifespan of the human population is continuously expanding, an increased understanding of the mechanisms that underlie the aging process is greatly needed. This is of importance not only to understand disease development in the context of age, but also with the long-term goal of eventually achieving an overall healthier state in the later stages of life [1], an objective that can be anticipated to be achieved through reversal, or at least inhibition, of the age-driven decay in organismal performance.

Although multiple attempts toward formulating more universal theories on the causes of aging have been put forward, the aging of multicellular organisms is undoubtedly a progressive multiparameter process [2] that is characterized by asynchronous/segmental phenotypes of various organs [3]. Despite this, a growing consensus supports an association of increasing age and a failure to appropriately maintain organ and tissue homeostasis or to return to homeostatic conditions following stress or injury [4]. Tissue-resident stem cells, which by now have been identified in most adult organs and tissues [5], have been suggested to be causally linked to the aging process [4]. From the standpoint that the primary function of stem cells is to maintain tissue homeostasis by replenishing cells lost through various insults, a contribution of stem cells to the aging process appears intuitive [5]. This, not the least, because age-associated mutational events or other forms of macromolecular damage gained at the level of somatic stem cells is at risk for propagation to its differentiated progeny. Ultimately, this can be envisioned to compromise either the generation or the function of the differentiated end products.

THE MANIFESTATION OF HSC AGING AND ITS RELEVANCE FOR THE AGING IMMUNE SYSTEM

One organ that critically depends on adult stem cell function is the blood, or hematopoietic, system [5]. In this system, all blood cells originate from rare bone marrow (BM)-residing, self-renewing hematopoietic stem cells (HSCs) that are capable of initiating a stepwise and hierarchical differentiation cascade. This involves the generation of various intermediate progenitor cell types with progressively narrowed differentiation potential, in which the final outcome is the generation of mature effector blood cells belonging to one of several distinct lineages [5].

At the population level, it is well established that the elderly have decreased potential to mount effective adaptive immune responses [6]. Although altered effector cell functions obviously

can contribute to such phenotypes, it is becoming increasingly clear that this is also the result of decreased production of naïve B and T lymphocytes with age. These latter shortcomings stem from alterations at multiple stages of hematopoiesis; various lymphocyte progenitor compartments decrease in abundance with age [7–9], with the remaining lymphocyte progenitors being functionally compromised [7, 10], which jointly leads to a reduced diversity of the B-cell repertoire [6, 11]. For T cells, thymic atrophy is undoubtedly a major contributor to reduced T lymphopoiesis with age [12]. However, more recent studies have illuminated that the reduced production of naïve lymphocytes with age also depends on cell intrinsic alterations in aged HSCs, made perhaps most evident by the observation that transplantation of aged HSCs into young recipient animals regenerates a blood system with aged properties, including reductions in T-cell output [8, 13–21]. The reduction of B- and T-lymphocyte production with age alters the cellular composition of the hematopoietic system, resulting in a relative dominance of myeloid cells, a phenomenon frequently referred to as myeloid skewing or bias [8, 13–21].

The overall blood-forming potential of aged HSCs was previously misconceived to be roughly equal to that of its young counterpart, because transplantation of young and aged unfractionated bone marrow cells into young recipient hosts yielded similar levels of reconstitution [18]. However, a consistent finding has been that the number of immunophenotypically defined murine HSCs increase on average 5- to 20-fold within the aged bone marrow [8, 13, 14, 16, 18, 19, 22–24], although the expansion of HSCs with age can vary several logs in between individual animals [14]. When taking this into account and instead comparing the blood-forming potential of young and aged HSCs on a per cell basis, aged HSCs are severely compromised in their ability to engraft recipient mice and to give rise to both lymphoid and myeloid progeny [13, 14, 25]. Perhaps the increased numbers of functionally compromised murine HSCs in the aged bone marrow may be interpreted as a futile compensatory response to maintain the production of naïve blood cells with increasing age. Because many of these findings were derived from observations in mice, an inevitable question is whether and to what extent they might be linked to the models used [26] rather than representing a characteristic commonly shared by other species, including humans. Clinical observations have suggested donor age as a negative factor on the treatment outcome of allogeneic bone marrow transplantation [27, 28], although one more recent report failed to find support for this notion [29]. However, it can often be difficult to evaluate whether HSC performance itself plays a major role in the dismal outcome when using older donors from such studies. This is because bone marrow transplantation using older donors is often confounded by other factors, in which especially graft-versus-host disease is a significantly contributing factor [27–29]. Further, although compromises in lymphopoiesis with increasing age have clearly been established in murine studies [7–10, 13–21], it is less clear that donor age itself adversely affects lymphoid recovery following transplantation in humans [30, 31]. In autologous bone marrow transplantations, increasing age negatively affects treatment outcome and reconstitution kinetics [32]. In this scenario, however, it needs to be kept in mind that the recipient is often heavily pretreated, which could subsequently cause greater toxicity in the elderly recipient, with reconstitution dynamics affected by HSC cell-extrinsic mechanisms. Collectively, it has therefore been difficult to establish a relationship between HSC aging and compromised performance in the setting of clinical bone marrow transplantation. However, in an alternative approach, a few recent studies have focused more directly on characterizing purified HSCs obtained from normal individuals. Data obtained from such work have suggested more similar overall phenotypes to those observed in mice [33, 34], suggesting that several of the observations obtained from mice might be considered an evolutionary trait conserved among different mammalian species.

When taking into account not only that the prevalence of various hematological diseases increase dramatically with age [35], but also that they predominantly arise from the myeloid lineages [9], it is tempting to speculate that an age-associated myeloid skewing might underlie several clinically relevant phenotypes, such as reduced adaptive immune competence and the increase in prevalence of several myelogenous diseases (Fig. 1). Directly supporting this interpretation are findings that the *BCR/ABL* fusion oncogene, which often is causative for both chronic lymphoid and myeloid leukemia, was only capable of giving rise to myeloid leukemia when assessed in an aging context [9]. Along the same line, *MLL*-rearranged leukemia associated with infants was shown to involve lymphoid progenitors with different immunoglobulin/T cell receptor-rearrangement patterns when compared with those occurring later in life [36], and, in fact, in both *MLL*-rearranged and *BCR-ABL*-positive leukemia, age is an important prognostic factor for survival [37].

The HSC compartment has for a long time been considered homogenous, with all HSC clones possessing equal blood cell-forming potentials. More recent data have to a large extent changed this view by suggesting that the HSC pool is made up of HSC clones with alternate and distinct differentiation potentials [10, 16, 38–40]. In addition, it has become evident that the clonal composition of the HSC compartment becomes altered with age; HSCs with a balanced or lymphoid-biased differentiation potential become scarce, whereas myeloid-biased clones come to dominate the HSC pool in the aging scenario (Fig. 2) [14, 16, 19, 38]. Such data imply that a dominance of myeloid-biased HSCs underlies the age-associated myeloid skewing of the immune system and provide support for a "clonal selection" model of HSC aging (Fig. 2B). This model can to some extent be regarded as opposed to a "population shift" model, which rather argues that HSC aging is caused by a gradual functional decline of all HSC clones with age (Fig. 2A). However, because most if not all HSC subtypes become functionally altered/compromised with age, the current available data would argue that these two models are not mutually exclusive. Rather, the observed HSC aging phenotypes instead result from a "composite model," in which a selection for myeloid-biased HSC clones occurs concomitantly to an overall decline in blood cell formation potential of the entire HSC pool (Fig. 2C) [14, 16, 19, 38]. Consistent with this, observations made in the hematopoietic system of a 115-year-old healthy woman suggested that not more than two HSC clones were responsible for all blood cell production at the extreme age point evaluated [41].

Triggers of Aging

From an evolutionary perspective, aging might in general be considered to be the result of selective pressures. If so, aging should have one or more underlying genetic components that preserve the necessary resources for the greater population, through the removal of individuals that are past their reproductive prime [42]. However, aging has also been suggested to result from the accumulation of DNA mutations [43] and pleiotropic genes: genes that are advantageous early in life but disadvantageous with increased age [44]. Although it is becoming increasingly clear that environmental

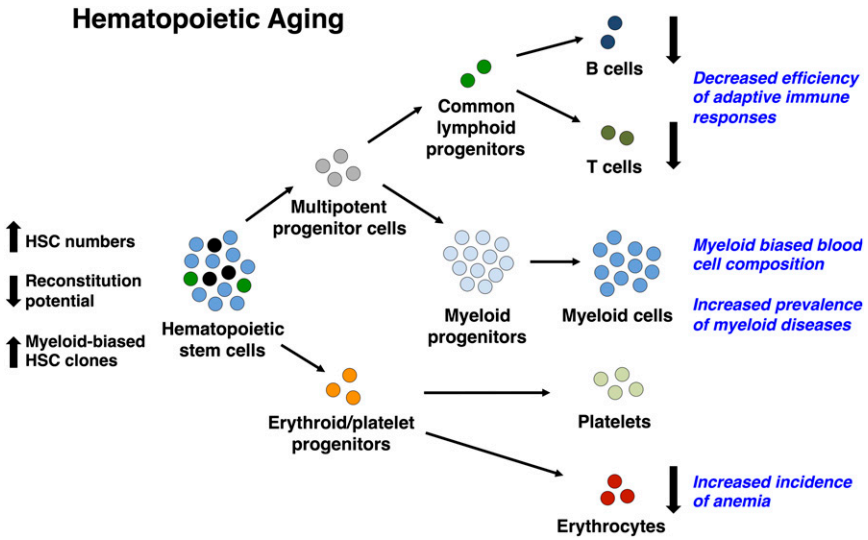

Figure 1. Age-associated alterations in the hematopoietic system. A number of immunological shortcomings arise as a consequence of advancing age and are thought to depend on cell intrinsic alterations occurring in aged HSCs for their maintenance and propagation. These, in turn, alter the composition of the hematopoietic hierarchy and overall blood cell output. HSCs also increase in numbers, which possibly reflects a compensatory response caused by their overall reduced blood-forming capacity. As the HSC pool becomes dominated by HSC clones with myeloid-biased differentiation potential (depicted as light blue HSCs), the abundance of balanced (depicted as black HSCs) and lymphoid-biased HSCs (depicted as green HSCs) decline with age. This results in a skewed blood cell composition, in which peripheral myeloid cells become more dominant, whereas both lymphoid progenitors and naïve B and T lymphocytes decrease in abundance. Abbreviation: HSC, hematopoietic stem cell.

factors impact on organismal fitness, the vastly different lifespan of many species in nature lends support to the notion that aging may be intrinsically governed [45]. It should be noted, however, that most animals in nature succumb to premature death, for instance from disease or predation, and generally do not exhibit human-like aging-associated characteristics [46]. In this regard, human aging, including the diminished immune responses with age, may be seen as an evolutionarily young "problem" that is the result of a prolonged life expectancy caused by an overall increased quality of life and improved health status.

Most investigations into HSC aging have been studied using mouse models, more specifically the C57Bl/6 mouse strain. Naturally, this is a concern for the general applicability of findings made and their translation to humans. Encouragingly, however, it has become increasingly clear that several aging phenotypes are conserved not only between alternate mouse strains [7–9], but also more importantly with human hematopoietic aging (Fig. 1) [10]. For instance, elderly humans were recently proposed to associate with an increased frequency of HSCs that harbors a myeloid-biased differentiation potential [10]. Other similarities to the mouse system include distinct decreases in the most primitive committed lymphoid progenitors and the frequent onset of mild anemia [11]. Thus, several key aspects of hematopoietic aging appear to be evolutionary and intrinsically conserved and, most importantly, can be studied using model organisms with shorter lifespans—a critical issue to experimentally establish causality.

Intracellular Alterations That Accompany HSC Aging

DNA Damage

HSCs must provide a lifelong supply of effector bloods and are therefore inherently very long-lived cells. As a consequence, HSCs have been proposed to be subject to increasing amounts of DNA damage and telomere erosion with age (Fig. 3A). The telomeres represent protective ends of the chromosomes that become shortened with each consecutive cell division and function to maintain chromosomal integrity [47]. Excessive telomere erosion leads to replicative senescence, at least in vitro [47], and late-generation telomerase-deficient mice that lack the enzyme capable of elongating and maintaining telomere length and therefore have critically shortened telomeres develop premature aging phenotypes [47]. However, nearly negligible telomere shortening has been observed when investigating telomere lengths in aged HSCs [13]. This might reflect the fact that HSCs, as opposed to most somatic cells, express detectable levels of telomerase [48], but also that these cells are mostly quiescent and infrequently undergo cell divisions [18, 49, 50]. However, excessive HSC cycling, as induced by repetitive transplantation, can lead to extensive telomere shortening that ultimately also associates with functional exhaustion [51]. Perhaps more unexpected was the finding that overexpression of telomerase in serially transplanted HSCs, which results in an appropriate maintenance of telomere length, did not protect HSCs from exhaustion [52]. Combined with the fact that inbred mice have significantly longer telomeres than humans [53], this argues strongly against telomere erosion as a primary mechanism of HSC aging and makes it less evident that therapeutic interventions aimed strictly at maintaining telomere length in aging HSCs would be an effective therapeutic avenue.

Other forms of DNA damage may arise as a consequence of replicative errors, oxidative stress, and environmental insults (Fig. 3A). Indeed, studies performed on mouse models that are compromised in various DNA damage repair pathways support a role for DNA damage in aging, because these models often develop premature HSC dysfunction and "aging-like" phenotypes

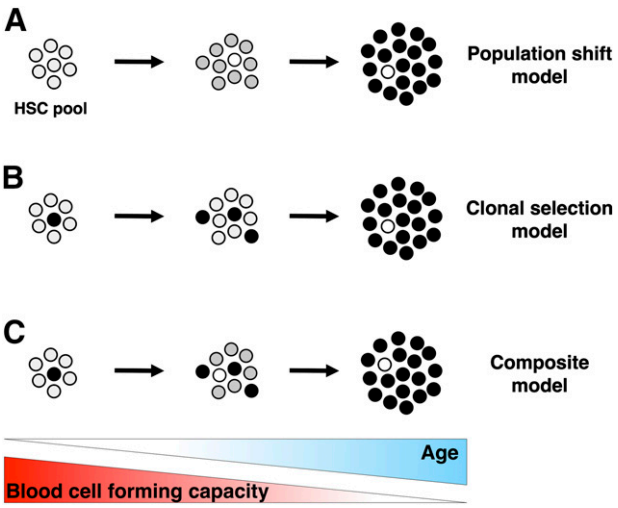

Figure 2. Models proposed to explain the reduced blood-forming capacity of the aged HSC pool. **(A):** The population shift model suggests that all HSCs are homogenous early in life (light gray circles) and that most HSC clones gradually (darker gray circles) become functionally altered with advancing age. This ultimately results in the manifestation of an aging state in which most, if not all, HSC clones are severely functionally compromised (dark gray circles). **(B):** The clonal selection model instead proposes that the HSC pool is heterogeneous and that increasing age results in a progressive expansion of selective HSC clones (depicted as an expansion of black circles). With advanced age, these HSC clones come to dominate the HSC pool, and few, if any, "young-like" HSC clones remain. **(C):** In the composite model, aging results in the selective expansion of certain HSC clones (black circles), which is accompanied by other HSC clones becoming functionally compromised with increasing age (dark gray circles). Upon advanced aging, the HSC pool consists of a high proportion of myeloid-biased HSCs and an overall drastically lowered blood-forming potential of most HSC clones. Abbreviation: HSC, hematopoietic stem cell.

[22, 54–59]. Moreover, when indirectly investigating the presence of nuclear DNA damage in aged HSCs by quantifying the presence of ϒH2AX foci, a surrogate marker for an active DNA damage response (DDR), both aged human and murine HSCs associate with an increased amount of DDR foci compared with their young counterparts [22, 60]. In a more recent work, aged HSCs were demonstrated to more directly associate with increased DNA damage [61]. However, in the same work, both young and aged HSCs were capable of appropriate DNA repair when forced into proliferation [61]. Thus, the quiescent nature of HSCs, which often is viewed as a protective feature for damage accumulation, may in fact be a fertilizer for progressive DNA damage accumulation with age. Alternatively, or in conjunction to this, highly quiescent HSCs lack the need to repair damaged DNA unless called into a new round of cell division. On the other hand, components induced by the DDR may have other roles in maintaining cellular function [62]. Therefore, although DNA damage accumulation alone might be unlikely to underlie the consistent phenotypes associated with HSC aging, an acute or chronic induction of transcriptional programs involved in DDR can perhaps also lead to an altered cell fate of aged HSCs. Should such DDR pathways prove to be causally involved in HSC aging, achieving a normalization of such responses might be therapeutically feasible. However, if different forms of nuclear DNA damage on its own are responsible for the age-associated decline, intervening with an established aging state would likely be doomed to fail, because this would require more extensive genome editing.

Cellular mitochondria, which uphold multiple key cellular functions such as the regulation of apoptosis and energy production, harbor genomes independent of the nuclear genome (mitochondrial DNA [mtDNA]) [63]. Reactive oxygen species (ROS) are normal by-products of active mitochondrial respiration and can inflict damage to various macromolecular cellular components, including DNA [64]. Because mtDNA reside in close proximity to the produced ROS [64] and because of the reliance on relatively crude DNA damage repair mechanisms compared with those acting on nuclear DNA [65], mtDNA has been proposed to be particularly prone to ROS-induced damage [65]. Building on this, the "mitochondrial theory of aging" proposes the continuous ROS production to result in a "vicious cycle" of mtDNA damage, further ROS production and a progressively lowered respiratory capacity that ultimately manifest into cellular aging [66]. Supporting this hypothesis are findings that mtDNA mutations accumulate in a number of aging tissues [67]. With this in mind, we recently investigated the relevance of mtDNA mutations in HSC aging using "mutator mice" that rapidly accumulate mtDNA mutations because of compromised quality control of the mtDNA replication machinery [15, 67]. Although the premature aging that occurs in these mutator mice coincides with defective lymphopoiesis and anemia, this occurred as a consequence of differentiation blocks at defined progenitor cells stages and impacted less directly on HSC function [15]. We suggest that this depends on the use of different metabolic pathways in stem cells compared with progenitors [68], with downstream hematopoietic progenitors acquiring a dependence on mitochondrial respiration for their appropriate generation and function. This is in fact highly similar to previous observations made on the differentiation of embryonic stem cells [69]. Therefore, although mtDNA mutations might accumulate in aged HSCs, persistent mtDNA mutations might be less likely to be major mediators of aging at the HSC level.

Transcriptional and Epigenetic Signatures of HSC Aging

Advances in cell isolation have allowed for isolation of distinct immature hematopoietic cell populations at high purity. By taking advantage of this, several studies have in recent years performed extensive gene expression profiling directly on young and aged HSCs [8, 13, 15, 23, 25, 61, 70, 71]. Collectively, these works have revealed that the gene expression patterns of young HSCs become distinctly altered with age. Interestingly, several genes that become upregulated with age are important for myeloid cell differentiation, whereas many downregulated genes associate with lymphopoiesis [8]. These findings provide molecular support for the age-associated myeloid skewing of the blood system and suggest that at least some of the phenotypes that arise during HSC aging have transcriptional underpinnings.

Distinct transcriptional programs are most often maintained by regulatory mechanisms at an epigenome level, because this ensures both their stability and their continuity. This also appears central for HSC aging, because alterations in gene expression observed in aged HSCs persist even following their transplantation into new hosts [13]. In addition, several components involved in chromatin organization and epigenetic maintenance have been reported to become dysregulated with age (Fig. 3B) [8, 23]. This suggests that both an altered transcriptome and epigenome contribute to the functional shortcomings associated with immunoaging. However, until very recently, investigations into the

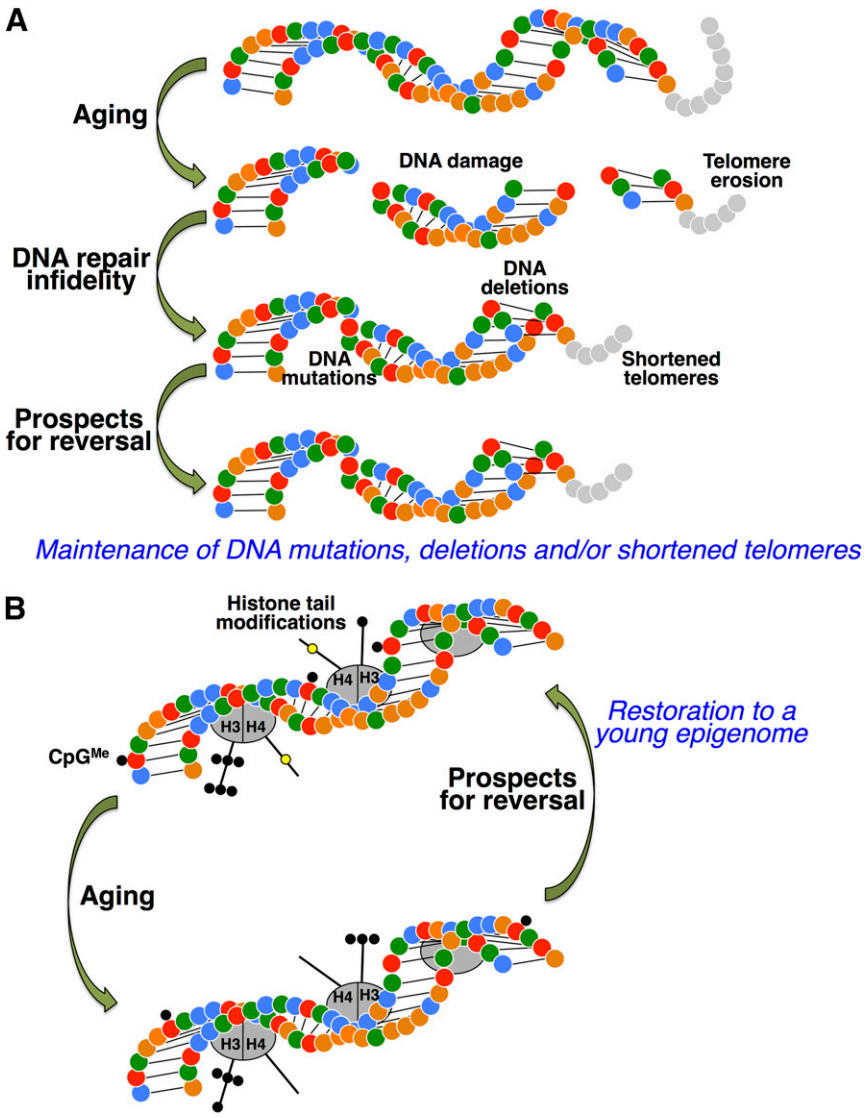

Figure 3. Molecular mediators of hematopoietic stem cell (HSC) aging and their consequences on reversal. **(A):** Because of their extensive longevity, HSCs have been proposed to be particularly prone to acquisition of age-associated DNA damage in the form of deletions, mutations, and/or telomere erosion. If DNA damage was the major driver of HSC aging, reversal strategies would be complicated by the irreversible nature of the changes. **(B):** Aged HSCs harbor epigenomic alterations in the form of altered distributions of DNA methylation and histone modifications, which underlie the altered transcriptome of aged HSCs. The reversal of such changes should hold the potential to functionally rejuvenate aged HSC function.

nature of the HSC aging epigenome have been restricted by the rare nature of HSCs, not offering sufficient material for direct study. A few reports have now circumvented this constraint [25, 71, 72]. This has led to the realization that aging HSCs display a distinct DNA methylome compared with their young counterparts. Sun et al. [71] found that the regulatory regions of many genes involved in HSC differentiation become hypermethylated, whereas genes involved in HSC maintenance become hypomethylated with age. This has provided molecular support for the observations that the overall mature effector cell output from aged HSC is decreased and might explain the expansion of the HSC pool in the aged setting. In addition, Beerman et al. [25] found that many hypermethylated promoter regions in aged HSCs are polycomb group (PcG) target genes. Interestingly, such locus-specific age-associated hypermethylation could be triggered by enforced and extensive HSC proliferation [25]. Therefore, although young and aged HSCs display a similar cell cycle status, most aged HSCs have undergone more cell divisions at a cumulative level, and it might be conceivable that HSC functionality becomes gradually altered with each consecutive cell division. Taiwo et al. [72] similarly observed a hypermethylation of PcG targets, and, because PcG genes function to maintain and propagate regulatory histone modifications [73], these findings corroborate in suggesting that an alteration of the histone "code" might also occur during HSC aging. In support of this, aged HSCs were found to inherit a differential occupancy of H3K4 and H3K27 trimethylation (usually considered to promote and repress gene transcription respectively) on a genome-wide scale, and reassuringly, age-associated H3K4me3 enrichment correlated with the transcriptional activities of these loci [71]. PcG proteins have

previously been recognized as being critically involved in the aging of various tissues, mostly because one well-known PcG target gene is the *Cdkn2a* locus that encodes a critical cell cycle regulating and senescence-inducing tumor suppressor product [74]. However, although *Cdkn2a* is induced in a number of tissues with age [75], HSCs appear to represent an exception, because *Cdkn2a* expression is virtually absent in both young and aged HSCs [76]. Although this provides at least some evidence against replicate senescence as being a major mechanism of HSC aging, PcG might target other loci involved in HSC aging. Elucidating the nature of such PcG targets will be important when striving both to understand HSC aging, but also when aiming to enhance aging HSC function. On this subject, the PcG maintenance gene *Bmi1* has been found to repress lymphopoiesis-associated loci in HSCs, and *Bmi1* loss results in their premature expression [77]. Moreover, Xie et al. [78] found that the loss of *Eed*, an accessory factor for both polycomb repressive complexes 1 and 2, results in HSC exhaustion. Florian et al. [17] also identified a global reduction of another histone mark, H4K16 acetylation, in aged HSCs. Finally, the impact of various noncoding RNAs to the aging process is so far a relatively untouched area [79].

Collectively, current data thus support that HSC aging strongly associates with a transcriptional and epigenetic "drift" (Fig. 3B), besides the potential role of DNA damage acquisition with age. However, although the contribution of epigenetics to the aging process is starting to be well established, one has to acknowledge the outstanding question of whether these changes are the drivers or rather the consequences of progressing age.

Is the HSC Aging State Reversible?

Interest into the transcriptional and epigenetic properties that define cell identity has been fueled by the demonstration that the introduction of embryonic stem (ES) cell-associated transcription factors into terminally differentiated somatic cells can revert their functionality into an ES cell state, a process accompanied by the erasure of the epigenetic parameters of the mature somatic cell [80, 81]. More recently, the prospects for more direct cellular reprogramming, from one somatic cell type into another, has been established [82]. From a hematopoietic perspective, reprogramming technologies hold great promise, because one limitation for bone marrow transplantation is the difficulty in finding suitable donors and, occasionally, sufficient HSCs. Therefore, attempts to generate transplantable HSCs by somatic cell reprogramming, either directly or by differentiating induced pluripotent (iPS) cells into HSCs, have been broadly explored. Although a few studies have managed to induce a multipotent hematopoietic program from fibroblasts and iPS cells [83–85], these studies have failed to generate transplantable HSCs with long-term function. This might be explained by the reliance on in vitro culture systems in such approaches, because HSCs cultured in vitro rapidly lose their stemness. Riddell et al. [86] recently acknowledged this problem and found that a brief exposure of terminally differentiated blood cells to a number of HSC-specific transcription factors in vivo could reprogram these mature cells into functional and transplantable HSCs.

Because the deregulation of even an individual transcription factor holds the potential to dramatically alter cell fate and identity, it appears plausible that HSC aging could result from the altered expression of certain key aging loci. If so, the normalization of such age-dysregulated loci should provide a means to functionally rejuvenate aging HSCs. We recently tested this hypothesis by first reprogramming aged hematopoietic stem and progenitor cells (HSPCs) into iPS cells and then redifferentiating these into HSCs in vivo using blastocyst complementation [13]. Interestingly, the function of the resulting HSCs was found to be highly similar to that of young HSCs and failed to resemble aged HSCs when investigated for several known age-related functional shortcomings [13]. Therefore, the HSC aging state appears to be reversible and primarily depend on an altered epigenome and transcriptome (Fig. 3B). In an extension, a fundamental question from both a basic scientific and an eventual therapeutic perspective is whether HSC aging is caused by the combinatorial action of many altered loci or whether one or few genes are responsible for the aging phenotypes. On this subject, Satoh et al. [87] found that aged HSCs express lower levels of the chromatin organizer *Satb1* than their young counterparts and that the overexpression of *Satb1* in aged HSPCs cells improved their ability to generate lymphoid progeny in vitro. The mitochondrial deacetylase *Sirt3* has also been proposed to have a role in HSC aging [88]. Specifically, *Sirt3* becomes downregulated in aged HSPCs, which was suggested to dampen cellular responses to ROS and oxidative stress [88]. Interestingly, the overexpression of *Sirt3* in aged HSCs resulted in decreased ROS levels and an increased reconstitution potential of all lineages. However, because the age-associated myeloid bias remained largely unchanged following *Sirt3* expression [88], this alone fails to explain this fundamental aspect of HSC aging. In conjunction with previous findings [15], this highlights the importance of proper mitochondrial function for appropriate blood formation. The mammalian target of rapamycin (mTOR) pathway, which integrates multiple signals from nutrients, growth factors, and oxygen to regulate critical cellular functions and has been implicated in organismal longevity [89], was found to exhibit an increased activity in aged HSCs [90]. Of note, providing aged mice with the mTOR inhibitor rapamycin resulted in a reduction of HSC numbers, an improved reconstitution potential, and a more balanced output of hematopoietic effector cells [90]. Thus, several phenotypes associated with HSC aging can be dampened by the administration of a single drug. Recently, loss of cell polarity was suggested to be another aberration occurring in aged HSCs [17]. In young HSCs, the distribution of the small Rho GTPase *Cdc42* is focal, whereas aged HSCs displayed both an increased abundance of the activated form of *Cdc42* and its more dispersed localization, with similar patterns observed also for other known polarity factors [17]. This loss of polarity has been proposed to depend on an age-associated *Wnt5a*-dependent shift from canonical to noncanonical Wnt signaling [91]. Remarkably, a brief exposure of aged HSCs to the *Cdc42* inhibitor *Casin*, followed by their transplantation, restored not only polarity but also dampened several phenotypes associated with HSC aging, including the myeloid bias and the expansion of the HSC pool [17]. In addition, because *Casin* treatment restored acetylated H4K16 to levels comparable to young HSCs, *Casin* treatment may, at least partly, be viewed as an epigenetic modulatory drug. These findings therefore emphasize not only a role for disrupted cell polarity in HSC aging but also reinforce the importance of an altered epigenome for maintaining the HSC aging state. Collectively, these studies provide support that individual gene products can have a strong influence on the emerging phenotypes associated with HSC aging, although their combinatorial actions are yet to be explored.

Although most data argue that HSC aging is a cell intrinsic phenomenon, HSC aging might also depend on, or be triggered by, extrinsic stimuli by either systemic factors and/or supportive cells in their immediate proximity. Perhaps supporting this interpretation, the demonstration of increased levels of the inflammatory cytokine Rantes in the aging BM microenvironment was suggested to contribute to the age-associated myeloid skewing [92]. In addition, we and others have found that the lack of the adaptor protein Lnk, which functions to dampen extrinsic cytokine signals, abrogates the phenotypes associated with HSC aging [93, 94]. Young as well as aged $Lnk^{-/-}$ mice display an expanded HSC pool. However, aged $Lnk^{-/-}$ HSCs harbored superior reconstitution potential compared with aged WT HSCs, with maintenance of a robust lymphoid differentiation potential [93, 94]. Recently, prolonged fasting was suggested to dampen the consequence of age on HSC function through normalization of the clonal distribution of the aging HSC compartment, that is, by reducing the frequency of myeloid-biased HSCs and increasing the frequency of lymphoid-biased HSCs [95]. These effects were attributed to reduced circulating IGF-1 levels and cellular protein kinase A activity, implicating nutrient signaling as a regulator of HSC aging [95]. Because a function of the mTOR complex is to mediate such signals, it would be interesting to establish whether the effects of rapamycin treatment and prolonged fasting on HSC aging share common mediators. Similar to the case of IGF-1, the supplementation of systemic GDF11 to aged mice was recently demonstrated to rejuvenate several aspects of the aging of other organ systems, including the brain, heart, and muscle [96–98]. This also might be an interesting avenue to investigate from an HSC aging perspective.

Although in many cases indirect, the findings described jointly argue that the aging environment and potentially the factors produced within it can also impact on the manifestation of the HSC aging state. However, as the transplantation of aged HSCs into a young environment reconstructs an aged hematopoietic system, cell intrinsic alterations in aged HSCs must be sufficient, at least for the maintenance of the physiological HSC aging state.

Future Perspectives, Therapeutics, and Challenges

Work conducted during the last decade has greatly extended our knowledge on the phenotypic representations of immunoaging and has started to unravel its underlying molecular mechanisms. Although we now know that DNA damage accumulates in aged HSCs, it is becoming increasingly clear that at least some aspects of HSC aging can be experimentally reversed. Therefore, epigenetic and transcriptional alterations, rather than DNA damage per se, appears to be key regulators of HSC aging (Fig. 3). Still, many questions remain unresolved. For instance, although a complete epigenetic reversal can rejuvenate aging blood cells, outstanding questions include whether this can be achieved also in a less invasive scenario and whether such approaches may be applied to rejuvenate human HSC aging. Although one might envision the use of already clinically used "epigenetic drugs" for this purpose, such as the histone deacetylase inhibitor valproic acid, it is not intuitive that results will be easy to interpret. Although the epigenome-wide action of such agents might rejuvenate age-dysregulated loci, they are also bound to alter epigenetic marks at many other genomic regions, resulting in a highly unpredictable outcome. Therefore, it would seem necessary to determine the exact nature of the deregulated loci in aged HSCs. Such regions could subsequently form the basis for more targeted rejuvenation therapies. Although to date limited, a few examples exist in which pharmacological modulation of deregulated factors in aged HSCs has been performed [17, 90]. Such interventions have, however, so far only achieved partial rejuvenation. Still, these studies represent encouraging proof-of-concept studies that modulation of the HSC aging state is possible via treatment of exogenous agents. In addition, with the assumption that we eventually will uncover a more complete knowledge of the appropriate targets, one might not only be able to intervene with an already established aged state, but perhaps also apply HSC aging-preventive treatments. To achieve this, it is important to distinguish modulation of aging within one organ from that applied to a whole individual. Because age progression is a multifactorial process, inhibition or even reversal of this process will likely require modulation of one or more crucial regulators of tissue homeostasis, which comes at a risk. For instance, an aging-promoting role of the tumor suppressor *Trp53* is well established [99], but interference with such a powerful tumor suppressor would most likely lead to the development of a cancer [100]. Further, if such therapies would ever become applicable in clinical practice, it would most likely be in the form of systemic therapy such as an "anti-aging pill." Because regulation of organ-homeostasis differs from one organ system to another, compound-based modulation could also be very organ-specific. Therefore, treatment with a compound to rejuvenate one organ could potentially lead to opposite outcomes in others. Perhaps illustrating this is the previous mentioned GDF11, which appears to have rejuvenating actions in brain, heart, and muscle [96–98]. However, the recent demonstration that GDF11 has inhibitory actions on erythroid development [101, 102] would postulate that treatment with such a compound could lead to hematological cytopenia.

Conclusion

The fact that HSCs are extremely rare cells continues to be a major experimental obstacle. Therefore, as techniques are developed and adapted to allow for studies on very infrequent cell populations, we anticipate that the detailed knowledge surrounding the molecular events that coincide with and drive HSC aging will continue to expand. For example, whereas the transcriptional landscape of aged HSCs have been relatively well defined, one area that remains relatively unexplored is the potential alterations of the proteome in aging HSCs. Finally, it must be stressed that as we uncover the identity of HSC aging regulators, the daunting challenge of investigating their interplay and physiological relevance should be undertaken. This is because studies directed at determining the involvement of candidate aging regulators often either ablates or increases the abundance of these candidates to nonphysiological levels, work that most often has been applied to a young setting. Although such approaches can be used to screen for candidate aging regulators, they fail to account for the contribution and crosstalk with other changes present in the aged cells. Such studies will be vital, because physiological HSC aging is caused by the combinatorial action of numerous intrinsic alterations, which likely explains why current targeted rejuvenation attempts have failed to completely rejuvenate aging HSC function. Therefore, to achieve full-fledged understanding of HSC aging, more holistic information will undoubtedly be critical to

thoroughly understand HSC aging and to eventually design appropriate rejuvenation approaches.

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Disclosure of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

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