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The aging hematopoietic stem cell niche: Phenotypic and functional changes and mechanisms that contribute to hematopoietic aging

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The hematopoietic system: the cellular hierarchy

In the classical model of hematopoiesis, HSCs sit at the apex of a developmental hierarchy. HSCs are self-renewing and give rise to all mature myeloid and lymphoid cell lineages. Although they are multipotent and self-renewing, HSCs are relatively quiescent in order to maintain an indefinite pool of HSCs [1]. The pool of HSCs contains multipotent progenitors (MPP), short-term HSCs (ST-HSCs), and long-term HSCs (LT-HSCs). The balance of HSC self-renewal with multi-lineage differentiation is critical for hematopoietic tissue homeostasis [2].

The first description of murine HSC-enriched cells came about with the introduction of monoclonal antibodies and fluorescent activated cell sorting (FACS) technology [2]. Murine HSCs are commonly identified and isolated by differential expression of individual combinations of cell surface markers [3]. HSCs in adult mice are all Lineage (Lin)[−], stem cell antigen-1 (Sca-1)⁺, and cKit⁺, often referred to as LSKs [2]. Murine LSKs, however, are diverse and can be further characterized with additional marker combinations. The most commonly used set of markers include the SLAM family of cell surface receptors that regulate the proliferation and activation of lymphocytes. They include CD150 (Slamf1), CD48 (Slamf2), CD229 (Slamf3), and CD244 (Slamf4) [3, 4]. These markers are differentially expressed among hematopoietic progenitors and correlate with progenitor

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primitiveness. This allows for adult LSKs to be subdivided into functionally distinct cell fractions containing HSPCs and MPPs [4]. Even more precise isolation of HSCs can be achieved by selecting for different LSK subsets including Thy-1^{low} [2, 5, 6], CD34⁻ [7], and Flt3⁻ [8-10] cells. Similar to murine HSCs, human HSCs can be divided into quiescent and self-renewing HSCs [11]. Human HSCs can be enriched using Lin⁻, CD34⁺, CD38⁻, Thy1.1⁺, CD45RA⁻, and in some cases CD49f⁺ [11].

The adult bone marrow also contains mesenchymal derived stem cells (MSCs) [12]. MSCs regulate skeletal remodeling and are multipotent progenitor cells that can mature into osteoblasts [13], adipocytes [14], myocytes, or chondrocytes. *In vitro*, MSCs can produce colony forming units of fibroblastoid colonies and differentiate into multiple lineages and support hematopoiesis [15]. *In vivo*, the osteogenic lineage has been linked with HSC expansion [16], while conditional ablation of osteolineage cells is associated with the loss of HSCs [17], demonstrating a functional role of osteolineage cells in hematopoiesis. Osteolineage cells also secrete cytokines vital for HSC regulation including granulocyte-colony stimulating factor [18], thrombopoietin [19], angiopoietin-1 [20], and CXCL12 [21] and can be manipulated with activation of parathyroid hormone receptor 1 [12, 16] or bone morphogenetic protein (BMP) receptor type 1A [22] to increase HSC function.

MSCs are phenotypically heterogeneous [23, 24]. MSCs can be identified with platelet derived growth factor receptor α (PDGFR α), CD51, and/or Sca-1 [25]. Nestin-GFP⁺ cells in the murine bone marrow are also multipotent and self-renewing MSCs [12]. MSCs can also be identified by other cell surface markers including CD105 [26, 27], CXCL12 abundant reticular (CAR) cells [28], and leptin receptor (LepR) perivascular cells [29, 30].

More recently, the HSC hierarchy has been reconfigured to include megakaryocytes [31, 32], particularly those with high cKit expression [33]. Within the myeloid lineage of the classical HSC model, bipotent megakaryocyte-erythrocyte and granulocyte-macrophage progenitors produce unipotent progenitors that ultimately give rise to all mature blood cells. This model has been challenged with the idea that high cKit expressing megakaryocytes may come directly from HSCs [34-36]. Two recent studies provide functional evidence for megakaryocytes in the murine HSC niche during both steady state and stress hematopoiesis [37] and in transplantation assays [38]. Additionally, *in vivo* megakaryocyte ablation results in a loss of HSC quiescence [39-41], providing further evidence for a megakaryocytic contribution to the HSC niche.

Extrinsic regulation of hematopoietic cells

Until recently, it remained poorly understood how HSCs could be influenced by extrinsic factors. It is now known that HSCs reside in the bone marrow surrounded by many cell types, including macrophages, vascular-forming endothelial cells, and cellular matrix proteins that can stimulate or inhibit hematopoietic niche function [35]. This is known as the hematopoietic niche or microenvironment and is essential for the production of blood forming elements, remodeling of the skeleton, and the maintenance of HSCs.

Technical challenges initially hampered the identification of dividing and quiescent HSCs within specific locations of the HSC niche [35]. HSCs are rare and only a few can be found in extremely thin tissue sections [3, 42] or upon live imaging [43]. However, advances in bone marrow imaging techniques, including whole-mount confocal immunofluorescence, computational modeling, and optical clearing methods, have demonstrated that hematopoiesis occurs and is regulated in anatomically distinct sub-microenvironments [44-47]. These paradigm-shifting imaging techniques are also supported by a range of definitions of patient-derived bone marrow cell populations [48-52], multiple isolation techniques [12, 53, 54], and the use of genetic mouse models [12, 55-57]. Given the complexity of cell types implicated in regulating hematopoiesis, it is likely that there are multiple hematopoietic niches. We will next review recent research demonstrating that HSCs reside in two anatomically distinct microenvironments that likely serve distinct functions. These microenvironments are identified herein as the arteriolar and sinusoidal microenvironments. However, it should be noted that, due to different experimental approaches used to localize HSCs in the bone marrow, it remains unclear whether these two microenvironments are truly distinct niches.

The arteriolar compartment is a highly vascularized, but hypoxic, microenvironment in which HSCs are thought to reside in a quiescent state [44, 46, 58]. This niche is composed of CD150⁺, CD48⁻, CD41⁻, Lin⁻ HSCs [44] as well as endothelial cells [3] and nestin-GFP^{bright} NG2⁺ arteriolar pericytes [55] that are distinct from sinusoidal-associated LepR⁺ cells [44]. NG2⁺ arteriolar pericytes express high levels of stem cell factor (SCF) and deletion of SCF from endothelial cells decreases HSC numbers [55]. Functionally, loss of NG2⁺ cells result in increased HSC cycling and decreased HSC-repopulating activity – hallmarks of HSC aging – and alters the distribution of HSCs from the NG2⁺ arteriolar compartment to the LepR⁺ sinusoidal compartment [44].

The arteriolar compartment also contains sympathetic nerves in the form of nonmyelinating Schwann cells that can regulate HSC behavior [59-61]. For example, nonmyelinating glial cells express HSC niche factor genes and are in constant contact with HSCs [61]. Sympathetic nerve denervation induces a rapid loss of bone marrow glial cells and a drastic reduction in HSC numbers, with the remaining HSCs identified as actively proliferating, indicating that sympathetic nerves function to maintain HSC dormancy in the central bone marrow. Sympathetic neurons are also involved in HSC mobilization as Katayama *et al.* demonstrated that mice that exhibit abnormal nerve activity lack HSC mobilization from the bone marrow following treatment with factors known to enhance HSC mobilization [59]. Likewise, in humans, catecholaminergic neurons promote CD34⁺ cell migration and engraftment [62]. This sympathetic nerve regulation of HSC maintenance is reported to be mediated indirectly by Nestin⁺ MSCs expressing β 3-adrenergic receptors [12]. Collectively, the identification of glial cells in the central bone marrow niche opens up an exciting new area of research that connects the hematopoietic and nervous systems.

Given the localization of HSCs near blood vessels, the vascular-forming endothelial cells are also critical for HSC homeostasis. Supporting a highly vascularized environment of the central bone marrow is the ability of circulating niche factors such as interleukin-8 (IL-8) to almost instantaneously mobilize HSCs [63]. Interestingly, the properties of niche-forming

blood vessels decreases with age but can be restored by Notch activation in endothelial cells [64].

Immunofluorescence studies with markers of primitive hematopoietic progenitors also suggest that HSCs reside in a sinusoidal compartment enriched with megakaryocytes, sinusoidal pericytes, and various stromal cell populations including CAR cells [28], LepR^+ [30], and $\text{nestin-GFP}^{\text{dim}+}$ stromal cells [45, 55, 57, 65]. In this niche, $\text{Nestin-GFP}^{\text{dim}+}$ cells overlap with LepR^+ cells and both of these populations contain MSCs [66]. Similar to the central bone marrow, cytokines play a significant functional role in HSC regulation as CXCL12 is strongly present in bone tissue [59] and genetic deletion of CXCL12 increases HSC mobilization [67]. Interestingly, new optical clearing techniques suggests that HSCs are in constant contact with bone associated LepR^+ and CXCL12^+ endosteal cells and that most HSCs are located in the sinusoidal compartment instead of near arterioles [45]. These data, which conflicts previous reports [44, 55], suggests that, with more research, the arteriolar and sinusoidal HSC niches could be subdivided into additional anatomically distinct domains [45].

Although it remains unknown how the two hematopoietic niches are affected with age, the presence of these distinct HSC niches implies important functional differences in regards to HSC homeostasis. It is conceivable that there is a continuous crosstalk between the arterial (quiescent) and sinusoidal (proliferative) compartments in order to tightly regulate hematopoiesis. Outstanding questions relate to the cellular complexity of these sub-niches, the role of the endosteum, and the functional heterogeneity among the arteriolar and sinusoidal microenvironments. Additional research into these anatomically distinct HSC microenvironments could uncover new and more precise mechanisms of HSC aging and, in turn, new and more precise targets to prevent or reverse HSC aging.

HSC functional changes with age

Hematopoietic aging is evident in the form of reduced adaptive immune responses [68-70], increased anemia [71, 72], and increased susceptibility to myeloproliferative diseases, including leukemias [73] and MDS [74]. HSCs accumulate cellular and molecular damage with aging [75] via multiple mechanisms. In this section we highlight the main phenotypes that are associated with HSC aging.

The number of phenotypically defined HSCs in the mouse bone marrow increases by two to tenfold with age [76-78]. This change in HSCs with age is highly strain dependent as aged C57BL/6 mice demonstrate two to threefold increases in HSCs [79] while HSC numbers are slightly decreased in aged DBA/2 and Balb/c mice [80-83]. This suggests that the self-renewal, differentiation, and quiescence of HSCs may be subject to significant genetic variation across mouse strains. Studies on human HSC aging also supports the view that phenotypic human HSC number (CD34^+ , CD38^+ , CD90^+) increases with age [84].

Even though murine HSCs increase with age, they appear to be dysfunctional [79, 85, 86]. In fact, per-cell self-renewing capacity declines with age [87]. The mechanistic basis for the observed functional decline remains unknown due to variations in the quantification and

purity of the HSCs assayed. It is suggested that aged HSCs are deficient in bone marrow engraftment such that when young and aged bone marrow cells are engrafted into a radioablated host and then retransplanted after twenty-four hours, the functional recovery from the recipient bone marrow is twofold lower for the aged HSCs [88]. This has been repeated in many other murine studies demonstrating that aged HSCs exhibit reduced repopulation capacity in competitive transplantation assays [76, 86, 89-91]. Similarly, HSCs isolated from the bone marrow of older human donors (>45 years of age) have reduced transplantation success in patients, indicating that human HSC self-renewal also decline with age [92].

Another age-associated phenotype of aged HSC donors and transplant recipients is their skewed differentiation towards the myeloid lineage [73, 93]. This phenomenon is consistent with the fact that pediatric hematopoietic malignancies are largely made up of lymphoid leukemias, while older individuals exhibit an observed increase in myelogenous diseases such as myelodysplastic syndrome (MDS) and acute myelogenous leukemia (AML) [73, 93]. This relative expansion of myeloid progenitors is considered a cell-autonomous function [78, 86]. However, with greater research investigating the influence of HSC niche interactions, it is not unreasonable to consider that niche factors may also contribute to skewed myeloid differentiation.

Aged HSCs also demonstrate enhanced mobilization from the bone marrow into the blood and reduced homing back to the bone marrow. Reports indicate that young and aged HSCs have distinct niche preferences *in vivo*, as suggested by the distinct anatomical positions of young and aged HSCs relative to the endosteum [85, 94]. Additionally, there is increased mobilization of aged HSCs to the blood in response to chemotherapy and cytokines compared to young HSCs [85, 86, 88, 95, 96]. Aged HSCs also have impaired adhesive properties [88, 96].

As HSCs require supporting cells from the surrounding niche to maintain proper function, it is plausible that the aged microenvironment also contributes to hematopoietic aging. For example, in other stem cell systems, heterochronic transplantation and parabiosis experiments show that stem cell aging is heavily influenced by cell-extrinsic mechanisms [97-99]. These mechanisms include increases or decreases in concentrations of circulating factors such as TGF β , IGF-1, and insulin and a failure of niche cells to properly transmit signals to stem cells [97]. These cell-extrinsic changes can ultimately affect HSC proliferation, differentiation, and cell fate decisions.

Mechanisms of HSC aging

While HSC aging has been extensively explored, the mechanisms of how aging alters the ability of the bone marrow microenvironment to support and maintain HSCs is only recently being investigated. New studies show that HSC aging is driven by both cell-intrinsic and cell-extrinsic mechanisms. In addition to altered transcriptional landscapes and abnormal transduction of signaling cascades that is observed in many aging processes, new concepts related to epigenetic modifications, cytoskeletal polarity, cellular senescence, and clonal

selection are also observed with aging. Below we provide a brief overview of the major cell-intrinsic and cell-extrinsic mechanisms that are likely to be involved in HSC aging.

Cell-intrinsic mechanisms

Altered transcriptional landscapes—Transcriptional profiles from young and aged HSCs have identified genes that correlate with HSC aging. There is a collective downregulation of lymphoid genes along with an upregulation of myeloid genes, such as runt-related transcription factor 1 (*Runx1*), *Hoxb6*, and *Osmr* in aged HSCs [76]. This is consistent with a skewing of aged HSCs towards myeloid differentiation. Molecules involved in cell-cell interactions, such as P-selectin and intercellular adhesion molecule 1 (ICAM1), and the nuclear factor- κ B (NF- κ B) response pathway are upregulated in aged HSCs compared to young HSCs, indicating increased inflammation in aged HSCs [100]. In support of an inflammatory phenotype, platelet integrin CD41 (α IIb β) also accumulates with age [93], and is associated with inflammation and platelet adhesion and activation [101].

In addition to traditional transcriptional profiles, several laboratories have reported changes in the global epigenetic profile of aged mouse HSCs [85, 102-104]. Correct processing of epigenetic information is critical for HSC self-renewal and is dynamically regulated by DNA and chromatin modifying enzymes. However, it remains inconclusive whether aging results in a more repressed or activated epigenetic profile [105]. For example, Beerman *et al.* found that DNA methylation of Polycomb Repressive Complex 2 (PRC2) by DNA methyltransferases (DNMTs) – an epigenetic marker associated with gene silencing – is increased in aged HSCs [102] while others demonstrate a bimodal pattern in DNA methylation status [106, 107]. In addition, genes involved in chromatin remodeling and chromatin-mediated transcriptional silencing have been shown to be downregulated in aged HSCs. These include histone methyltransferases, acetyltransferases, deacetylases, and SWI/SNF chromatin remodeling complexes. DNA accessibility genes such as sirtuins are also downregulated in aged HSCs [100]. These studies demonstrate that aged HSCs are vulnerable to DNA and chromatin dysregulation, which may contribute to the altered epigenetic status of aged HSCs [108] and to their functional decline and increased propensity for neoplastic transformation.

Cell polarity and CDC42—In addition to DNA and chromatin modifying enzymes, cellular polarity may also regulate the accessibility of genetic information [85, 109]. Cellular polarity refers to the distribution pattern of proteins, DNA, and RNA within a cell and has been implicated in HSC aging [85, 109]. Genome-wide association studies have shown increased levels of the small Rho GTPase cell division control protein 42 (CDC42) in both aged murine [85] and human [110] HSCs. The toggle of CDC42 between the GTP-bound active and GDP-bound inactive state occurs in response to various HSC stimuli, including growth factors, cytokines, and extracellular matrix interactions. Increased CDC42 activity results in a loss of cell polarity in aged HSCs [85] and in other stem cell niches such as epithelial [111], muscle [112], and neural [113] stem cells.

Alterations in DNA damage response and telomere shortening—The age-associated increases in hematological malignancies have been associated with shortened

telomeres and defective DNA repair mechanisms. In fact, decreased regenerative capacity of aged mouse HSCs is suggested to result from both an age-dependent shortening of telomeres [114] and an accumulation of DNA damage [115-117]. Telomeres are DNA sequences at the ends of chromosomes that prevent the activation of DNA repair mechanisms. Without telomerase – the crucial enzyme necessary to add nucleotides to the ends of telomeres – telomeres lengths are reduced after each round of cell division. Telomere shortening ultimately produces chromosomes that are vulnerable to DNA damage.

In support of a DNA damage dependent mechanism, aged mouse HSCs have increased levels of γ H2AX [116, 118, 119] and elongated tails in comet assays [117, 119, 120] – markers for double-stranded breaks and DNA damage, respectively – compared with young HSCs. Age-related DNA damage also accompanies physiological HSC aging in humans [121]. Interestingly, however, Moehrle *et al.* demonstrated that even though aged HSCs have increased DNA mutational burden compared to young HSCs, they respond to DNA damage in a similar fashion as young HSCs [119]. This finding raises doubts of whether aged HSCs actually have more DNA damage than young HSCs.

In support of a telomerase dependent mechanism, mice with dysfunctional telomerase – and thus shortened telomeres – show premature HSC aging [122]. In relation to disease, a genetic mouse model that contains eroded telomeres produces hallmarks of human MDS and predisposes common myeloid progenitors to differentiate towards the myeloid lineage [123]. However, similar to the DNA damage theory, the physiological importance of telomeres in HSCs is uncertain because mouse HSCs express low levels of telomerase and the overexpression of telomerase does not increase their self-renewal capacity in serial transplantation experiments [124]. It is also unclear if telomeres shorten sufficiently in HSCs during the lifetime of mice [125] and humans [126] to significantly contribute to an aging phenotype. Additional studies are needed to clarify the extent and contribution of DNA damage and telomere attrition to HSC aging.

Increased ROS production and oxidative damage—Oxidative damage is mediated primarily through the production of reactive oxygen species (ROS) and affects the replication and transcription of mitochondrial DNA (mtDNA), leading to decreased mitochondrial function. Decrease mitochondrial function in turn generates a cascade of increased ROS production and further mtDNA damage, ultimately resulting in cellular senescence and cellular aging.

HSCs are quiescent, have inherently a low metabolism rate, and generate low levels of oxidative stressors. However, upon aging, ROS levels can accumulate and result in ROS-induced oxidative free radical damage [127]. Transgenic mice that have increased mtDNA mutations as a result of a deficiency in the mitochondrial DNA polymerase catalytic subunit gamma (POLG) develop lymphopenia and anemia [128], indicating that mtDNA damage, ROS production, and hematopoiesis are related. Supporting this, HSCs from ROS^{low} mice retain their long-term self-renewing capacity, while HSCs ROS^{high} mice do not successfully transplant into recipient mice [129]. Furthermore, ROS^{high} mice treated with the ROS inhibitor *N*-acetyl cysteine (NAC) or with a p38 MAPKinase inhibitor were able to create colony forming units, further demonstrating a role of ROS and p38 MAPKinase in ROS-

mediated HSC homeostasis [129]. Interestingly, HSCs that have high levels of ROS also show increased activation of p38 and mammalian target of rapamycin (mTOR), which lead to the depletion of HSCs after serial transplantation [130]. However, additional research is needed to verify the causal role of mtDNA mutations and oxidative damage in contributing to HSC aging as data comparing ROS levels in physiologically aged HSCs and in HSCs from *Polg*^{-/-} mice show no change in ROS levels [131]. Despite these inconsistencies, there is a general consensus for an undefined role for mitochondrial mutations, ROS production, and oxidative damage in hematopoietic aging.

Clonality—The concept of clonality shifts is a new trademark of HSC aging and is associated with an increased risk of hematologic cancer [75]. HSCs are considered to be equipotent in their contribution to hematopoiesis with multiple clones contributing to hematopoiesis throughout life. Higher levels of clonality, an indicator that only a few clones actively contribute to blood cell production, are observed upon aging [132-134]. Sun *et al.* demonstrate that multiple clones contribute to hematopoiesis during development, but, later in adulthood, long-term HSCs – as opposed to HSCs – contributed to hematopoiesis [133]. Using limiting dilution transplantation experiments, Bush *et al.* similarly demonstrated that short-term HSCs increasingly contribute to hematopoiesis during aging [134]. By means of computational models, it is implied that the aged-associated myeloid bias may arise from reduced multipotent progenitor differentiation to common lymphoid progenitors rather a change in the HSC pool [134]. Using high-throughput sequencing to follow the clonal contribution to hematopoiesis, Verovskay *et al.* demonstrated that young mice have fewer active HSC clones that readily self-renew, but aged mice have more active HSC clones with less self-renewal capacity [132].

Cell-extrinsic mechanisms

Although much of the functional decline that occurs with HSC aging is cell-intrinsic, it would be careless to ignore the influence of the surrounding HSC niche. New and exciting data reveal important roles for HSC niche derived extrinsic factors in contributing to the HSC aging process. Moreover, and discussed in the following section, in other systems such as muscle stem cells, restoration of niches or sensitization of aged stem cells to extrinsic signals contributes to their rejuvenation. Here we summarize recent data demonstrating the relevance of changes in niche composition and function that occur with aging.

The bone marrow niche interacts with HSCs to orchestrate their survival, proliferation, self-renewal, and differentiation [16, 22, 59, 135, 136]. As discussed above, HSC niches are composed of many cell types and their fate are additionally regulated by many secreted factors. Age-induced alterations in niche composition include decreased bone formation, increased adipogenesis, and changes in extracellular matrix components. New competitive transplantation assays in which young and aged bone marrow cells have been transplanted into young and aged mice demonstrate that the aged HSC microenvironment partially contributes to the skewing of HSCs towards the myeloid lineage, through secretion of the pro-inflammatory CC-chemokine ligand 5 (CCL5) [137]. This niche-induced skewing of HSC differentiation may promote disease progression in acute myeloid leukemia [138] and myelodysplastic syndrome [74].

CXC-chemokine ligand 12 (CXCL12) is another critical chemokine in the bone marrow niche. It is a chemoattractant for HSCs, regulating their localization, turnover, and mobilization from the bone marrow. With age, there is increased fat content in the bone marrow attributed to the differentiation of bone marrow mesenchymal stem cells into adipocytes. This increase in fat content is inversely correlated with CXCL12 plasma levels in the elderly and directly correlated with the increased HSC numbers also observed in the elderly [139].

Together, these studies demonstrate a critical crosstalk between HSCs and their microenvironment. As a result, alterations in niche composition, niche interactions, and diminished or abnormal communication between HSCs and their environment may drive HSC aging. As discussed above, remaining areas of research involve a more complete characterization of anatomically-dependent sub-niches along with the cellular and functional complexity of each sub-niche.

Reversibility of aging phenotypes

Novel findings in which differentiated cells can be reprogrammed into induced pluripotent stem cells (iPSCs) suggest that – in addition to HSC differentiation – HSC aging may be a two-way street. When iPSCs derived from aged murine HSCs were dedifferentiated back into HSCs and behaved similar to young HSCs [140], it was inferred that the aging process may be reversible. As HSC aging is regulated by many cell-intrinsic and cell-extrinsic mechanisms, investigators are questioning whether or not these age-associated changes are reversible with clinically significant functional consequences. As such, many avenues are being explored to either directly target aged HSCs (cell-intrinsic) or to indirectly target the aged HSC niche (cell-extrinsic).

Epigenetic programming is one heavily studied cell-intrinsic avenue to dampen the HSC aging process. For example, sirtuins are mitochondrial histone deacetylases and are reduced in aged mouse HSCs. Overexpression of *Sirt3* [141] or *Sirt7* [142] has been reported to rescue aging-associated HSC defects. *Satb1* is another epigenetic regulator of lymphoid progenitors [143] and is reduced in aged mice. Overexpression of *Satb1* via epigenetic reprogramming rescued aged HSC immunosenescence [143]. These studies, among many others, demonstrate the availability of multiple epigenetic targets to dampen or possibly reverse the effects of HSC aging.

In relation to pathway targeting, CDC42 [85] and mTOR [144] are activated in aged HSCs, making them attractive pharmacological targets for HSC rejuvenation. Pharmacological inhibition of both CDC42 and mTOR activity have been shown to be sufficient in reversing age-associated HSC phenotypes [85, 144]. For example, *ex vivo* treatment of aged murine HSCs with the CDC42 inhibitor, CASIN, suppressed CDC42 activity to the levels found in young murine HSCs and reversed multiple age-associated phenotypes [85]. In addition to regulating CDC42 activity, H4K16Ac levels are decreased in aged HSCs and CASIN treatment elevated H4K16Ac levels to those of young HSCs, further emphasizing the importance of the epigenetic landscape in aged HSCs. Likewise, mTOR inhibition with rapamycin reversed the aged-associated increase in HSC numbers and reconstitution potential and self-renewal in aged mice [144]. Furthermore, CDC42 and mTOR are also

implicated in longevity in humans [110] and mice [145], making them even more promising targets to reverse HSC aging.

HSC aging can also be pharmacologically reversed in mice with antioxidative therapy [130] and with caloric restriction [146-148]. Caloric restriction prolongs the lifespan in both invertebrates and vertebrates [147] and can restore the aging-associated skewed myeloid differentiation and the reduced repopulation capacity of aged mouse HSCs [148]. Moreover, caloric restriction was reported to prevent myeloid leukemia in an irradiation-induced mouse model of leukemia [149]. Importantly, these strategies highlight the critical and indispensable relationship HSCs have with their surrounding niche for proper hematopoiesis. This critical relationship emphasizes that additional investigation into sub-anatomical niche targets could lead to new and clinically relevant strategies to dampen or restore hematopoiesis in the elderly.

Collectively, evidence in murine models suggests that the HSC aging process may be reversed pharmacologically or physiologically by targeting both cell-intrinsic and cell-extrinsic factors that contribute to aging. By doing so, the rejuvenation and reprogramming of HSCs may improve the adaptive immune response in aged populations and therefore reduce the risk for age-associated myeloid malignancies and immune dysfunction. Rejuvenating agents may also have applications for the treatment of bone marrow from aged donors to improve transplantation success rates. However, many questions remain unanswered. How do rejuvenating agents affect aged lymphoid progenitors? Do rejuvenating agents reverse clonal expansion in aged HSCs? Do rejuvenating agents influence the aged microenvironment? Does their effectiveness differ in the central marrow vs. bone associated niche? Thorough investigation of these outstanding questions could increase our understanding of HSC aging and uncover novel therapeutic strategies to effectively reverse or slow the aging process.

Concluding remarks

With the numerous intrinsic and extrinsic mechanistic theories to explain the process of hematopoietic aging, it is important to keep in mind the challenges that leave major questions related to HSC aging unanswered. Additional undefined roles of the HSC microenvironment in regulating hematopoiesis are foreshadowed by the functional redundancy between various cell populations that support HSCs and the novel inclusion of megakaryocyte precursors in the hematopoiesis hierarchy [33]. Region-specific HSC niches [44, 150] also pose new questions into the role of the HSC niche in driving HSC aging and disease. Technical concerns surrounding the heterogeneity of experimental approaches to localize HSCs [151] and the heterogeneity of aged HSC phenotypes among mouse strains [80, 152, 153] also pose challenges to studying the aged hematopoietic system. Nevertheless, a more detailed understanding of HSC aging and identifying circumstances under which aged HSCs may become functionally similar to young HSCs represents the first step in discovering future treatments for age-related hematopoietic diseases. Such scientific progress would be expected to impact not only hematopoietic cells employed in stem cell transplantation, but also hematopoietic malignancies and acquired marrow failure syndromes.

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