



Microenvironmental contributions to hematopoietic stem cell aging

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ABSTRACT

Hematopoietic stem cell (HSC) aging was originally thought to be essentially an HSC-autonomous process, which is the focus of another review in the same issue of *Haematologica*. However, studies on the microenvironment that maintains and regulates HSC (HSC niche) over the past 20 years have suggested that microenvironmental aging contributes to declined HSC function over time. The HSC niches comprise a complex and dynamic molecular network of interactions across multiple cell types, including endothelial cells, mesenchymal stromal cells, osteoblasts, adipocytes, neuro-glial cells and mature hematopoietic cells. Upon aging, functional changes in the HSC niches, such as microenvironmental senescence, imbalanced bone marrow mesenchymal stromal cell differentiation, vascular remodeling, changes in adrenergic signaling and inflammation, coordinately and dynamically influence the fate of HSC and their downstream progeny. The end result is lymphoid deficiency and myeloid skewing. During this process, aged HSC and their derivatives remodel the niche to favor myeloid expansion. Therefore, the crosstalk between HSC and the microenvironment is indispensable for the aging of the hematopoietic system and might represent a therapeutic target in age-related pathological disorders.

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Introduction to hematopoietic stem cell aging

Adult hematopoiesis takes place in the bone marrow (BM), where hematopoietic stem cells (HSC) can self-renew, proliferate and differentiate to replenish the blood and immune systems. Given that most HSC are quiescent under homeostasis, mature blood and immune cell production is believed to derive mainly under steady state from progenitor cells (rather than HSC), which differentiate to produce mature blood cells. Cumulative studies have demonstrated that HSC are heterogeneous and contain subsets with distinct myeloid, platelet or lymphoid-biased potentials, although the existence of lymphoid-biased HSC has long been debated and remains controversial.¹⁻⁵ Additionally, recent studies have shown that HSC can bypass the intermediate steps to generate mature progenies under certain conditions, such as chronic inflammation and aging.

Upon aging, HSC increase in number but their functions are impaired, characterized by reduced regenerative and homing capacity, loss of cell polarity, and myeloid-biased differentiation at the expense of lymphopoiesis.⁶⁻⁹ These changes were initially thought to cause only cell-intrinsic dysregulation,¹⁰ such as epigenetic deregulation,¹¹ replication stress,¹² deficient DNA repair,¹³ and transition from canonical to non-canonical Wnt signaling.¹⁴ Old HSC also suffer metabolic changes,^{15,16} impaired autophagy¹⁷ and altered protein homeostasis,¹⁸ which contribute to the decline of their regenerative potential. However, current studies are revealing that the BM microenvironment may contribute to HSC aging. This hypothesis is supported by an elegant study in which old HSC transplanted into young recipients exhibited reduced myeloid output as compared those transplanted into old recipients, suggesting that the old BM microenvironment contributes to myeloid skewing.¹⁹ This review will cover microenvironmental contributions to

HSC aging, provide hypotheses for BM niche remodeling based on current knowledge, and discuss the potential implications for age-related myeloid malignancies. HSC-intrinsic aging mechanisms are the focus of a separate complementary review in this issue of *Haematologica* and will not be discussed here.

Evolving views on hematopoietic stem cell niches

HSC are surrounded by numerous cell types and the associated extracellular matrix in the BM, which form a unique microenvironment known as the “HSC niche”. Osteoblasts were the first niche cells found to be involved in hematopoiesis. Early studies indicated that osteoblasts differentiate from BM osteoprogenitor cells, secrete hematopoietic cytokines and can maintain HSC in culture.²⁰ In 2003, two studies described for the first time that transplanted HSC localize to the bone surface of BM and their numbers are regulated by osteoblastic cells. Long-term HSC were found to adhere to spindle-shaped N-cadherin⁺CD45⁻ osteoblastic (SNO) cells, which control HSC size by BMP signaling.²¹ A recent study has shown that N-cadherin⁺ cells maintain a population of highly quiescent reserve HSC,²² suggesting the possibility that different BM niches might regulate steady-state vs. stress hematopoiesis. Another study showed that osteoblasts activated with parathyroid hormone/parathyroid hormone-related protein receptor produce high levels of Notch ligand Jagged 1 and increase HSC numbers.²³ Later studies further identified Tie2/angiopoietin-1 signaling and thrombopoietin/MPL signaling as important regula-

tors of HSC quiescence through interactions with osteoblasts.^{24,25} A high calcium concentration in the endosteum also plays an indispensable role, maintaining HSC in the endosteal niche, since calcium-sensing receptor knock-out HSC fail to migrate to the endosteal BM surface after transplantation.²⁶ In addition, the endosteal BM area is enriched in CXCL12²⁷ and stem cell factor,²⁸ two of the most important molecules supporting hematopoiesis, strengthening the hypothesis that the endosteum is a major reservoir for HSC. However, the osteoblastic niche was thereafter challenged in studies in which osteoblastic-specific deletion of Cxcl12 or Scf only affected the maintenance of early lymphoid progenitors but had little impact on HSC.²⁹ Furthermore, N-cadherin expression in osteolineage cells seems to be dispensable for HSC maintenance under homeostasis.³⁰ Whereas N-cadherin might not be essential for HSC, N-cadherin⁺ cells appear necessary to maintain a reservoir population of quiescent HSC.²² Studies on these aspects raise the possibility that different niches might exist for activated/quiescent HSC, and/or for HSC contributing to steady-state/emergency hematopoiesis. The BM is highly vascularized, and the close developmental relationship between hematopoietic and endothelial lineages together suggest that HSC are housed and regulated in perivascular regions. To date, at least two functionally distinct perivascular niches that highly express Cxcl12 and Scf to dictate HSC cell fate have been identified in mice: (i) the arteriolar niches, composed mainly of arterioles (found throughout the BM) or endosteal transition-zone vessels, both of which are associated with sympathetic nerve fibers, Nestin-GFP^{bright} and/or NG2⁺ cells; and (ii) the sinusoidal niches, where sinusoid-associated Cxcl12-abundant reticular cells,

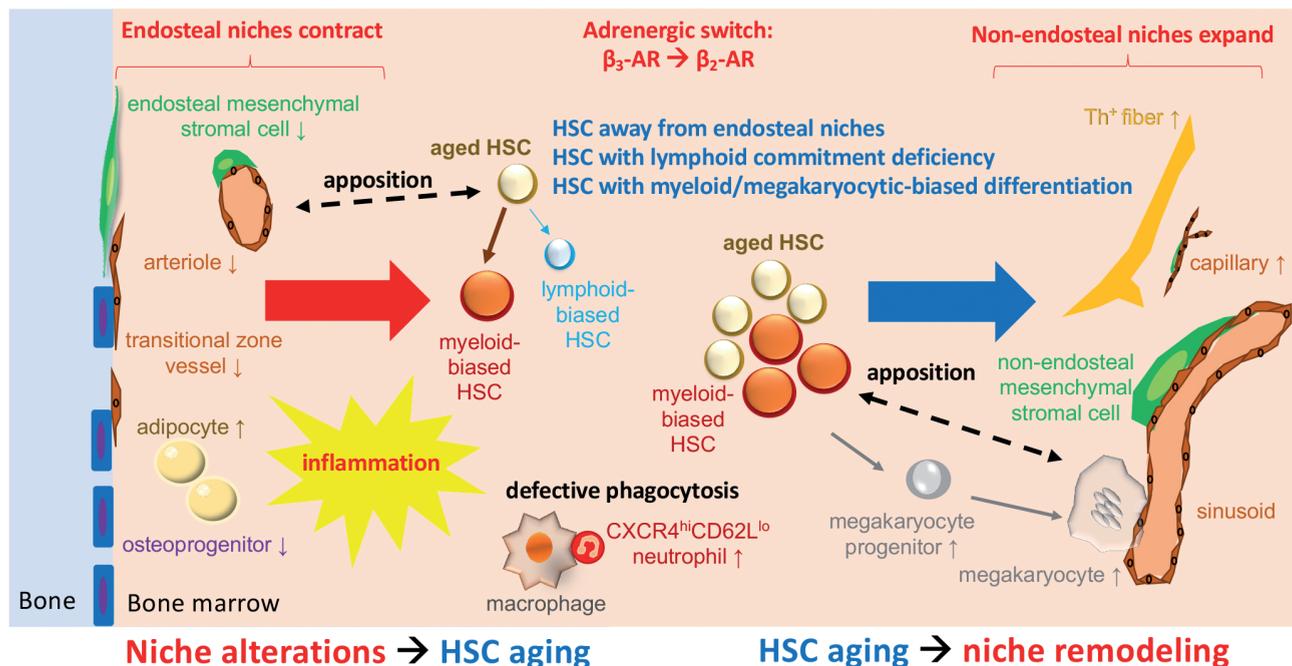


Figure 1. Schematic model of the interplay between hematopoietic stem cells and the microenvironment during aging. Loss of β_3 -adrenergic receptor (β_3 -AR) activity reduces endosteal niches, pushes hematopoietic stem cells (HSC) away from the endosteum and favors myeloid bias at the expense of lymphopoiesis. Accumulation of aged HSC in the central bone marrow and increased β_2 -AR activity causes expansion of central capillaries, myeloid cells and megakaryocytes, which locate farther from HSC.

Nestin-GFP^{dim} and LepR⁺ cells are located.³¹ Recent studies also reveal that megakaryocytes, which are mostly adjacent to sinusoids, regulate HSC quiescence through transforming growth factor- β , thrombopoietin and platelet factor-4 secretion.³²⁻³⁴ Currently, it remains controversial which specialized niches predominantly regulate HSC quiescence. It is possible that HSC quiescence is differently regulated between steady-state and emergency and/or malignant hematopoiesis. However, lineage commitment appears to be influenced by the location of HSC and their derivatives in the BM. Accumulating evidence suggests that lymphopoiesis preferentially occurs near the endosteum, while myelopoiesis/erythropoiesis/megakaryopoiesis mostly takes place in non-endosteal BM regions. Supporting this concept, a recent study using *Vwf-eGFP* to label different HSC populations demonstrated that *Vwf*⁺ platelet/myeloid-biased HSC are associated with megakaryocytes, whereas *Vwf* lymphoid/unbiased HSC are located close to arterioles.³⁵ Therefore, alterations in specialized niches might directly affect myeloid/lymphoid output, and the imbalanced production of mature hematopoietic cells at specific niches might in turn remodel the local microenvironment for these cells.

Hematopoietic stem cells change location as niches are remodeled during aging

A growing body of evidence has indicated that HSC redistribute within the BM upon aging. For instance, aged HSC locate away from the bone surface (endosteum), compared with young HSC, upon BM transplantation.³⁶ This abnormal homing behavior correlates with increased BM HSC numbers and enhanced HSC egress into the circulation.³⁷ Recent studies using whole-mount immunofluorescence staining of murine long bones further revealed that aged HSC are more distant from the endosteum, arterioles, Nestin-GFP^{high} cells and megakaryocytes, but HSC distance from sinusoids and Nestin-GFP^{low} cells appears unchanged, compared with that of young HSC.³⁸⁻⁴⁰ These results strongly suggest that the BM microenvironment is altered with age, favoring HSC lodging near non-endosteal (central) niches, over endosteal niches. The following sections will discuss current studies on age-related BM niche remodeling, the key microenvironmental players and the associated mechanisms by which HSC localization and function are regulated.

Dysfunction of bone marrow mesenchymal stromal cells

Studies regarding the absolute number of BM mesenchymal stromal cells (MSC) during aging have yielded controversial results, with some suggesting an overall increase,^{41,42} while others suggest unchanged^{43,44} or reduced numbers.⁴⁵ It is noteworthy that BM MSC are heterogeneous, and the heterogeneity in the markers used to define BM MSC immunophenotypically might explain some of these controversies. Using *Nestin-gfp* to label murine BM MSC, different studies have reported reduced endosteal Nestin-GFP⁺ cells in the aged BM,^{39,40} consistent with reduced numbers of arteriolar α SMA⁺, PDGFR β ⁺ and NG2⁺ cells.³⁸ The age-related contraction of endosteal BM might initiate lymphoid deficiency, since lymphoid niches have been previously described near bone.^{29,46-48} However,

this notion has been refined more recently after elucidating dynamic interactions between B-cell progenitors and perivascular BM MSC, which provide key signals for B lymphopoiesis (such as Cxcl12 and Il7), both in endosteal and central sinusoidal BM niches.⁴⁹⁻⁵² Functionally, old BM MSC exhibit reduced colony-forming unit-fibroblast (CFU-F) capacity *in vitro* and reduced expression of HSC niche factors.³⁸ In this regard, revitalizing BM MSC to restore HSC niche factors has been proposed as a strategy to prevent DNA damage in cultured HSC.⁵³

BM MSC exhibit reduced osteogenesis with age, which is associated with lower osteopontin secretion to the extracellular matrix.⁵⁴ Osteopontin negatively regulates HSC proliferation,⁵⁵⁻⁵⁷ and its decline might accelerate HSC divisions during aging. Supporting this idea, treatment with thrombin-cleaved osteopontin partially reverses the age-associated phenotype of HSC.⁵⁴

CC-chemokine ligand 5 (CCL5), a pro-inflammatory cytokine involved in bone remodeling,⁵⁸ is reportedly increased with age. Researchers also reported a direct contribution to myeloid-biased differentiation at the cost of T cells by CCL5,¹⁹ suggesting that CCL5 is important for aging of the hematopoietic system and the microenvironment. In contrast, old BM MSC show adipocyte skewing.⁵⁹ Adipocytes are a BM niche component that promotes HSC regeneration after irradiation, although their roles in hematopoiesis under homeostasis seem to be dispensable.⁶⁰ However, altered functions of adipose tissue, including ectopic lipid deposition, insulin resistance and increased inflammation, have been described during aging.⁶¹ Accumulation of BM adipocytes upon aging not only reduces hematopoietic reconstitution, but also disrupts bone fracture repair.⁶² The latter likely contributes to the increased risk of osteoporosis and bone fracture in the elderly population.^{63,64}

BM aging is also associated with senescence of BM MSC, evidenced by increased p53/p21-mediated DNA damage, upregulation of p16(INK4a) and elevated levels of reactive oxygen species.⁶⁵⁻⁶⁷ An age-dependent shortening of telomeres was found in telomerase-deficient (*Terc*^{-/-}) BM MSC; consequently, lethally-irradiated *Terc*^{-/-} mice carrying wildtype BM cells display accelerated myelopoiesis.⁶⁸ More recently, proteome analyses of human BM have unraveled nitric oxide synthesis and the urea cycle pathways as potential mediators for the crosstalk between old BM MSC and HSC.⁶⁹ Murine BM MSC show comparatively higher mRNA expression of neuronal nitric oxide synthase (encoded by the *Nos1* gene), as compared with other nitric oxygen synthase isoforms, and *Nos1*^{-/-} mice develop certain features of premature aging, such as remodeled BM vasculature and myeloid skewing.³⁹ Given the importance of nitric oxide in vascular biology and balanced inflammatory responses, it is likely that nitric oxide pathways participate in the aged vascular remodeling and myeloid expansion partly by modulating inflammation.

Remodeling of bone marrow vasculature and endothelial cell functions

During aging, remodeling of the BM endothelial vasculature is notable. Studies using whole-mount confocal imaging, two-photon intravital microscopy and flow cytometry analysis demonstrated overall increased vascular density in aged mice.^{38,70} Yet distinct vascular beds show different, or even opposite alterations with age. Arterioles appear to be decreased, while sinusoids seem

Table 1. Microenvironmental players contributing to hematopoietic stem cell aging.

Players	Size	Functions/Mechanisms
Mesenchymal lineages	Decreased endosteal Nestin-GFP ⁺ cells Increased non-endosteal Nestin-GFP ⁺ cells Decreased α SMA ⁺ cells, NG2 ⁺ cells, PDGF β ⁺ cells	Increased adipogenesis Decreased osteogenic differentiation Cellular senescence Altered nitric oxide, urea cycle pathways Reduced secretion of niche factors HSC closer to non-endosteal niches, away from endosteal niches
Endothelial cells	Increased overall vascular density Decreased arterioles; shortened arteriole segments Unchanged/preserved sinusoids Decreased transitional zone vessels Increased small capillaries	Increased vascular leakiness Vasodilation Decreased angiogenic potential Decreased Notch activity HSC away from arterioles HSC preserved in sinusoids DLL4 regulating HSC myeloid skewing Sinusoidal Jag2 regulating HSC proliferation
Inflammatory cytokines	Increased IL-1, IL-3, IL-6, TNF α , INF γ , TGF β	IL-1 β regulating HSC myeloid-skewing IL-6 regulating megakaryocyte differentiation TNF α protecting HSC from necroptosis TGF- β regulating megakaryopoiesis IFN regulating megakaryocytic bias
Sympathetic nervous system	Increased Th ⁺ nerve fibers	β_2 -AR activation regulating HSC myeloid-skewing toward platelet production β_2 -AR inactivation regulating niche remodeling, HSC lymphoid deficiency Functional switch of β adrenergic signaling (β_2 -AR overriding β_3 -AR)
Others	Increased megakaryocytes Accumulation of CXCR4 ^{high} CD62L ^{low} senescent neutrophils	Megakaryocytes closer to sinusoids HSC away from megakaryocytes Macrophages with impaired phagocytosis

GFP: green fluorescent protein; α SMA: alpha smooth muscle actin; PDGF β : platelet-derived growth factor; HSC: hematopoietic stem cells; IL: interleukin; TNF α : tumor necrosis factor alpha; INF γ : interferon gamma; TGF β : transforming growth factor beta; DLL4: delta-like 4; Jag2: Jagged 2; AR: adrenergic receptor.

unchanged upon aging.³⁹ Consistent with these observations, arteriole segments covered by Nestin-GFP^{high} cells appear shortened.³⁸ Transitional zone vessels containing type-H endomucin (EMNC)-high endothelial cells (which are enriched in the murine trabecular BM, where they support developmental bone growth⁷¹) are reduced in old mice.^{39,70} In contrast, small capillaries (CD31^{high}EMCN⁻ cells <6 μ m in diameter) are notoriously expanded in the central marrow.³⁹

The functionality of vascular endothelium declines with age, as manifested by increased vascular leakiness, increased levels of reactive oxygen species and decreased angiogenic potential.⁷² Poulos *et al.* previously reported that HSC purified from young mice and co-cultured with endothelial cells from old mice lack long-term hematopoietic multilineage reconstitution, while old HSC co-cultured with young endothelial cells maintain their self-renewal ability.⁷² Infusion of young endothelial cells into aged, conditioned mice revives the old hematopoietic system. Kusumbe *et al.* identified high Notch activity in type-H endothelial cells and their associated subendothelial/perivascular cells,⁷⁰ suggesting that contraction of endosteal vessels upon aging concomitantly occurs with impaired Notch signaling. Overexpression of the Notch ligand Dll4 in vascular endothelial cells can prevent myeloid skewing of hematopoietic progenitors⁷³ but cannot completely rescue HSC aging,⁷⁰ perhaps consistent with the finding of another study in which Dll4 was unchanged in the aged murine BM.⁴⁰ A common finding is reduced endosteal activity of Notch ligand, since the latter study reported reduced expression of Jagged2 (Jag2) ligand in aged Nestin-GFP^{high} cells. In contrast, Jag2 levels seem

increased in the sinusoids, or their associated Nestin-GFP^{low} cells. Moreover, Jag2 blockade induces proliferation and clustering of aged HSC near the sinusoids. Therefore, whereas the specific role of Dll4 during aging is not clear, alterations of Notch signaling do seem to be important for hematopoietic aging. Together, these results strongly suggest that altered Notch signaling critically contributes to HSC aging in different ways depending on the niche: in the endosteal vessels, Notch signaling appears to regulate HSC lineage commitment, whereas it is required in the sinusoids to preserve old HSC (since HSC accumulate in sinusoidal niches as a function of age).⁴⁰

Inflammation

Aging of the BM microenvironment is associated with increased pro-inflammatory cytokines, both in mice and humans.⁷⁴ Several lines of evidence have indicated that these inflammatory cytokines drive myeloid/megakaryocytic differentiation. In aged-related myeloid malignancies, such as myeloid proliferative neoplasms and chronic myelogenous leukemia, serum interleukine (IL)-1 β and IL-6 levels are elevated.^{75,76} Pietras *et al.* reported that chronic IL-1 exposure induces HSC myeloid skewing at the expense of self-renewal.⁷⁷ IL-1 α/β regulates thrombopoiesis *in vitro*,^{78,79} possibly explaining high platelet counts in aged mice.⁸⁰ Defective phagocytosis of macrophages during aging induces expansion of platelet-biased HSC through IL-1 β signaling.⁸¹ IL-6 promotes thrombopoiesis either through a direct effect on BM megakaryocyte differentiation³⁹ or indirectly upregulating hepatic

thrombopoietin levels.⁸² Yamashita *et al.* demonstrated that transient stimulation of tumor necrosis factor- α prevents HSC from necroptosis, and proposed that constitutive activation might lead to hyperproliferation of HSC and exacerbated myelopoiesis in aging and myeloproliferative disorders.⁸³ Megakaryocytes express transforming growth factor- β to regulate HSC quiescence, while megakaryocyte-derived transforming growth factor- β also stimulates thrombopoietin synthesis by BM stromal cells to enhance megakaryopoiesis.⁸⁴ An elegant study by Haas *et al.* found that acute inflammation induces proliferation of the stem cell-like megakaryocyte progenitor to quickly replenish platelet loss, and the process is in part mediated through the interferon family.⁸⁵

Despite the well-known lymphocyte deficiency associated with aging, only the frequency and function (but not the absolute number of lymphoid-biased/balanced HSC) appear to decline with age.⁸⁶ In fact, during aging both platelet/myeloid-biased and lymphoid-biased HSC expand, but exhibit altered gene expression programs and myeloid and platelet-skewing.⁸⁰ These findings suggest a cell fate change of HSC upon aging, and the net outcome is an increase of the myeloid/platelet compartment at the expense of the lymphoid compartment. Two possible non-mutually exclusive explanations are: (i) different HSC suffer the same intrinsic abnormalities upon aging and (ii) microenvironmental alterations specifically influence HSC and direct their cell fate. In support of the second possibility, distinct HSC subpopulations respond differently to inflammatory challenges during aging.⁸⁷ Moreover, old lymphoid-biased/balanced HSC seem to retain normal lymphoid commitment potential when removed from an old microenvironment.⁵ Exogenous addition of IL-1 can block lymphocyte differentiation from old lymphoid-biased HSC, confirming the indispensable role of IL-1 in HSC fate decisions. Consistently with this notion, IL-1 blockade seems sufficient to revert the age-dependent increase of megakaryocytic-biased HSC *in vitro*.⁸¹ IL-1 blockade can also attenuate myeloid expansion and inflammatory arthritis associated with the elderly.⁸⁸

However, whether the inflamed BM niche is the cause or the consequence of HSC aging remains debated. It is notable that mature myeloid/megakaryocytic cells are a major source of inflammatory cytokines.⁸⁹ Therefore, exacerbated myelopoiesis during aging might induce myeloid/megakaryocytic HSC skewing through inflammatory remodeling of the BM microenvironment. Many different cytokines directly affecting HSC (e.g., IL-6, tumor necrosis factor and interferon) increase during aging. A positive feedback loop between the myeloid cells and their derived inflammatory cytokines might increase both myelopoiesis and the cytokine storm. Future studies are needed to clarify the roles of other inflammatory cytokines, such as IL-6, in the regulation of HSC during aging.

Neuronal regulation by sympathetic adrenergic signaling

It has been reported that BM sympathetic stimulation of β_2 or β_3 adrenergic receptors (AR) regulates the egress and granulocyte – colony-stimulating factor -induced mobilization of HSC.⁹⁰⁻⁹² A recent publication has suggested that noradrenergic nerve fibers are reduced in old murine

BM.⁸⁸ The study also indicated that surgical denervation of young BM induces premature aging of the hematopoietic system, although the inflammation caused by the surgical denervation might have had a certain influence. A similar reduction of nerve fibers was found in a mouse model of an age-related blood disorder, myeloproliferative neoplasm,⁷⁵ suggesting that BM neuropathy might predispose to myeloid malignancies with age. However, a recent study did not find such a reduction of BM noradrenergic fibers in aged mice.⁹³ Moreover, whole-mount imaging and three-dimensional reconstruction of different bones has revealed doubled BM area covered by noradrenergic nerve fibers in aged mice.³⁹ This result is consistent with the well-known increase in sympathetic activity in the elderly, manifested for instance by an increased concentration of noradrenaline in the human plasma with age.⁹⁴⁻⁹⁷ Increased sympathetic activity causes myeloid skewing of old HSC through activating β_2 -AR, since exacerbated thrombopoiesis is present in old wildtype mice but absent in old *Adrb2*^{-/-} mice or *Adrb2*^{-/-}*Adrb3*^{-/-} mice.³⁹ A previous study showed that α -AR directly regulated megakaryocyte migration, adhesion and proplatelet formation under stress, but α -AR did not affect the earlier commitment of progenitor cells to the megakaryocyte lineage.⁹⁸ Therefore, the age-dependent increase in sympathetic innervation might activate different AR as hematopoietic progenitor cells differentiate along the megakaryocyte lineage. Additionally, the sympathetic nervous system is known to control inflammation in a context-dependent manner.⁹⁹ The concentration of catecholamines and the levels of expression of different AR influence the inflammatory state of innate immune cells. Increased sympathetic activity during aging might contribute to the cytokine storm by activating inflammatory cells, and subsequently affect lineage-bias of HSC.

Interestingly, β_3 -AR exhibits opposite effects on lymphomyeloid skewing, compared with β_2 -AR. Adult *Adrb3*^{-/-} mice show a decreased frequency of endosteal lymphoid-biased HSC and/or lymphoid multipotent progenitors.^{38,39} Age-related remodeling of vasculature, such as reduced transitional zone vessels and expanded small capillaries, possibly explains the lymphoid deficiency in these mice.³⁹ Altogether, these results suggest that lack of β_3 -AR in the microenvironment might accelerate aging of the hematopoietic system. One study suggested that administration of a β_3 -AR agonist to old mice rejuvenates most features of HSC aging, but overall changes of hematopoiesis in peripheral blood were not reported in this study.³⁸ The same β_3 -AR agonist reduced myeloid expansion in a mouse model of myeloproliferative neoplasm⁷⁵ and a murine model of Hutchinson-Gilford progeria syndrome (HGPS).³⁹ However, hematopoietic rejuvenation was not detected in the peripheral blood of mice treated with this β_3 -AR agonist over 40 weeks in another study.⁷⁵ Likewise, elderly individuals with myeloproliferative neoplasms who received a β_3 -AR agonist over 24 weeks did not show rejuvenation in the peripheral blood counts in a human study.¹⁰⁰ Five-month old mice lacking β_3 -AR reportedly showed normal peripheral blood counts³⁹ or premature, lymphoid deficiency and myeloid skewing.³⁸ The discrepancies between these studies suggest that modulating a single neuronal pathway might not be sufficient to rejuvenate overall hematopoiesis. This may be due to the multiple compensatory/adjustment mechanisms of the autonomic nervous system revealed, for instance, in the cardio-

vascular system.¹⁰¹ It is worth mentioning that myeloid and lymphoid cells are also a source of catecholamines.¹⁰² Adrenal gland-derived adrenaline and immune cell-derived (nor)adrenaline might contribute to increased levels of adrenaline and noradrenaline in the circulation of aged individuals⁹⁴⁻⁹⁷ which, together with the increased BM noradrenergic innervation, might activate BM AR. We propose that sympathetic regulation of lympho-myeloid skewing pivots on activation or inactivation of different AR. A functional switch of neurotransmission (β_2 -AR overriding β_3 -AR) with age, rather than a general decline of BM noradrenergic innervation,³⁸ might initiate BM niche remodeling and subsequently promote HSC myeloid skewing toward platelet production. Further investigation of other adrenergic and/or cholinergic signaling pathways possibly influencing aging is warranted.

Other players in the bone marrow microenvironment

Emerging data suggest that the progeny of HSC can feed back to regulate their activity under homeostasis, raising the possibility that mature hematopoietic cells (or their interactions with others) also contribute to HSC aging. For instance, clearance of senescent CD62L^{low}CXCR4^{high} neutrophils by macrophages has been reported to modulate HSC niches.¹⁰³ Frisch *et al.* discovered that aged macrophages are unable to engulf senescent neutrophils, leading to expansion of megakaryocytic-biased HSC through IL-1 β signaling.⁸¹ Another key player is the megakaryocyte, reportedly expressing CXCL4, thrombopoietin and transforming growth factor- β to control HSC proliferation/quiescence.³²⁻³⁴ In murine BM, around 20% of HSC are spatially associated with megakaryocytes,³² and depletion of megakaryocytes expands platelet-biased HSC.³⁵ During aging, megakaryocytes are found in increased numbers, lodging closer to sinusoids, and abundantly forming pseudopodial extensions (likely to be proplatelets).³⁹ Of note, the distance between HSC and megakaryocytes increases substantially,^{38,39} suggesting a remodeling of megakaryocyte niches with age. It is possible that megakaryocytes with these morphological changes fail to anchor HSC, inducing HSC hyperproliferation and lineage bias. However, whether and how age-related alterations of megakaryocytes regulate HSC aging is still unknown and requires further investigation.

Premature aging in Hutchinson-Gilford progeria syndrome

In HGPS, aberrant splicing of the *LMNA* gene (encoding lamin A and C) leads to nuclear assembly of the truncated protein, prelamin A (progerin).^{104,105} Certain hallmarks of murine hematopoietic aging, such as increased platelet counts, have been observed in HGPS.¹⁰⁶ Given that cells aging naturally also express increased levels of progerin,¹⁰⁷ it is possible that normal physiological conditions and progeria might share some aging mechanisms. Grigoryan *et al.* recently reported that HSC deficient of LMNA display a premature aging-like phenotype,¹⁰⁸ suggesting a prominent role of lamin A/C in hematopoiesis at the level of HSC. The strong impact of progeria on growth and sexual maturation might be paralleled by altered endocrine regulation of HSC, since growth hormones and sex hormones regulate HSC survival, proliferation and lineage commitment.¹⁰⁹⁻¹¹² However, it remains unknown whether

premature hematopoietic aging in HGPS is a consequence of progerin accumulation in HSC, other hematopoietic cells and/or the microenvironment. Using the *Lmna*^{G609G/G609G} mouse model,¹¹³ we found that primary *Lmna*^{G609G/G609G} mice exhibit features of premature hematopoietic aging; however, premature hematopoietic aging is not observed in wildtype recipients carrying *Lmna*^{G609G/G609G} hematopoietic cells.³⁹ Microenvironmental alterations are observed in *Lmna*^{G609G/G609G} mice, some of which are shared between normally aged mice, such as elevated levels of pro-inflammatory cytokines (IL-3, IL-6, IL-1, interferon- γ), increased megakaryocytes with proplatelet-like structure and megakaryocyte apposition to BM sinusoids. Of note, β_3 -AR agonism improves exacerbated myeloid expansion and restores the apposition of HSC to megakaryocytes. These results suggest that premature hematopoietic aging in HGPS is not HSC-autonomous, and certain aging features can be rejuvenated by targeting the microenvironment.³⁹

Aged hematopoietic stem cells/ microenvironment: chicken or egg?

One remaining key question relates to whether microenvironmental alterations initiate HSC aging and/or whether old HSC cause niche remodeling. It is noteworthy that HSC aging is not characterized by a single cellular feature, and different aging features might emerge individually at different developmental stages. For instance, in murine aging, defective lymphopoiesis starts early, at the age of 8 months old,¹¹⁴ while increased platelet counts do not seem to be pronounced until 18 months.³⁹ BM noradrenergic nerve fibers appear to be decreased in adult, 8-month old mice⁷⁵ (Supplementary Figure 5B in the report of that study), but these fibers appear increased in aged (20-month old) mice, when β_3 -AR signaling is already strongly reduced.³⁹ A deficiency in β_3 -AR accelerates the loss of endosteal lymphoid-biased HSC in 4-month old mice, but it does not aggravate HSC aging in old mice.³⁹ In contrast, a deficiency in β_2 -AR impairs megakaryopoiesis in young and old mice, and double knockout mice for β_2 -AR and β_3 -AR recapitulate the hematopoietic phenotype of single β_2 -AR-deficient mice. These results suggest that β_2 -AR overrides β_3 -AR during aging, and that this adrenergic remodeling contributes to imbalanced lymphoid/myeloid output. We propose that a lack of β_3 -AR activity near endosteum initiates the contraction of endosteal niches, which attenuates lymphopoiesis, favors myeloid bias and pushes HSC away from the endosteum. An adrenergic switch from β_3 -AR to β_2 -AR could feed back to worsen the reduction of endosteal niches, since activation of β_2 -AR on osteoblasts is known to restrain bone formation.¹¹⁵ However, the cell types expressing β_2 -AR and/or β_3 -AR involved in aged hematopoiesis are still elusive. Chances are that cells highly expressing β_2 -AR replace those with high expression of β_3 -AR over time, or BM niche cells ubiquitously increase β_2 -AR while decreasing β_3 -AR upon aging. These and other hypotheses need to be validated in future studies¹¹⁶ since the experiments were performed using global knockouts. Tissue-specific deletion of β_2 -AR and/or β_3 -AR will provide further insights into the mechanisms of β -adrenergic switching during aging. Importantly, β_2 -AR and β_3 -AR have different affinities for noradrenaline and adrenaline; whereas β_3 -AR

shows higher affinity for noradrenaline over adrenaline, the opposite is true for β_2 -AR.¹¹⁷ Another possibility might be that BM concentrations of both neurotransmitters differ in aging, leading to imbalanced stimulation of the two receptors. The observation that old HSC home in the BM away from endosteal regions suggests that HSC-driven niche remodeling mainly occurs in non-endosteal domains. For instance, skewed myelopoiesis leading to increased numbers of neutrophils and defective phagocytosis of marrow macrophages might modulate the microenvironment favoring myeloid bias during aging.⁸¹ Increased myeloid cells might provide an additional source of catecholamines in a feed-forward loop promoting megakaryocyte differentiation by activating β_2 -AR. We propose that accumulation of old HSC results in microenvironmental remodeling by reinforcing adrenergic activity, expansion of non-endosteal niches and enhanced myeloid/megakaryocyte differentiation. As a secondary outcome, alteration of megakaryocyte niches (increased megakaryocyte numbers, proplatelet formation and apposition to sinusoids) might release HSC from their state of quiescence, further promoting HSC proliferation with age.

Niche alterations might predispose to hematologic neoplasms

The risk of developing myeloid malignancies increases significantly in individuals harboring clonal hematopoiesis-related somatic mutations. In fact some of these mutations are oncogenic drivers of myeloid malignancies.¹¹⁸ However, the factors limiting clonal expansion or, instead, allowing the mutant clones to become dominant and, in some cases, cause disease, remain unclear. Interestingly, similar niche alterations are shared between aging and myeloid disorders associated with age, which is

an important selection pressure to expand aberrant HSC clones. For instance, a damaged neuro-MSC circuit promotes the development of a cytokine storm created by the mutant HSC, aggravating the progression of myeloproliferative neoplasms.⁷⁵ In myelodysplastic syndromes, abnormal production of cytokines from the microenvironment, dysfunction of MSC and osteolineage cells, and vascular remodeling have been associated with disease initiation and progression.^{119,120} Targeting the abnormal microenvironment could be a promising complementary therapeutic approach to treat hematologic cancers in the future, especially when early diagnosis becomes available.

Conclusive remarks

Aging of the hematopoietic system might result from both HSC-intrinsic and microenvironmental alterations with changes in the location, function and regulation of HSC and their progeny. Future studies will determine the relative contribution of the aged microenvironment to altered hematopoiesis and increased incidence of age-related disorders originating in the BM.

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