Microenvironmental contributions to hematopoietic stem cell aging

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Running title: niche alterations promoting hematopoietic aging

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 HSCs (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, bone marrow mesenchymal stromal cells (BMSCs), osteoblasts, adipocytes, neuro-glial cells and mature hematopoietic cells. Upon aging, functional changes in the HSC niches, such as microenvironmental senescence, imbalanced BMSC differentiation, vascular remodeling, changes in adrenergic signaling and inflammation, coordinately and dynamically influence the fate of HSCs and their downstream progeny. The end result is lymphoid deficiency and myeloid skewing. During this process, aged HSCs 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.

Introduction to HSC aging

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

Upon aging, HSCs 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 HSCs also suffer metabolic changes (15, 16), impaired autophagy (17) and altered protein homeostasis (18), which contribute to the decline of their regenerative potential. However, current studies reveal that the BM microenvironment may contribute to HSC aging. This hypothesis is supported by an elegant study whereby old HSCs transplanted to young recipients exhibit reduced myeloid output as compared to 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 on 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 the hematopoietic stem cell niches

HSCs are surrounded by numerous cell types and the associated extracellular matrix in the bone marrow (BM), which form a unique microenvironment known as ‘’HSC niche’’. Osteoblasts were the first niche cells found to be involved in hematopoiesis. Early studies indicated that osteoblasts differentiated from bone marrow (BM) osteoprogenitor cells secrete hematopoietic cytokines that can maintain HSCs in culture (20). In 2003, two studies described for the first time that quiescent HSCs preferentially

locate near the bone surface of the BM after transplantation and that HSC numbers are regulated by osteoblastic cells. Long-term (LT) HSCs 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 HSCs (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 (PPRs) produce high levels of Notch ligand Jagged 1 and increase HSC number (23). Later studies further identified Tie2/angiopoietin-1 signaling and thrombopoietin/MPL signaling as important regulators of HSC quiescence through the interaction with osteoblasts (24, 25). High calcium concentration in the endosteum also plays an indispensable role maintaining HSCs in the endosteal niche, since calcium-sensing receptor (CaR) knockout HSCs fail to migrate to the endosteal BM surface after transplantation (26). In addition, endosteal BM area is enriched in CXCL12 (27) and stem cell factor (SCF) (28), two most important hematopoietic supporting molecules, strengthening the hypothesis that the endosteum is a major reservoir for HSCs. However, the osteoblastic niche was thereafter challenged in studies where the osteoblastic-specific deletion of Cxcl12 or Scf only affects the maintenance of early lymphoid progenitors but has little impact on HSCs (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 HSCs, N-cadherin^{+} cells appear necessary to maintain a reservoir population of quiescent HSCs (22). These studies raise the possibility that different niches might exist for activated/quiescent HSCs, and/or for HSCs contributing to steady-state vs. emergency hematopoiesis.

The BM is highly vascularized, and the close developmental relationship between hematopoietic and endothelial lineages suggests that HSCs are housed and regulated in perivascular regions. To date, at least two functionally distinct perivascular niches highly expressing Cxcl12 and Scf have been identified in mice: 1) 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; 2) the sinusoidal niches, where sinusoid-associated Cxcl12-abundant reticular cells (CAR), Nestin-GFP^{dim} and/or LepR^{+} cells are located (31). Recent studies also reveal that megakaryocytes, which are mostly adjacent to sinusoids, regulate HSC quiescence through TGFβ, TPO and PF4 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 HSCs and their derivatives in the BM. For

instance, accumulating evidence suggests that lymphopoiesis preferentially occurs near the endosteum, while myelopoiesis/erythropoiesis/megakaryopoiesis mostly takes place in non-endosteal or central BM regions. Supporting this concept, a recent study using *Vwf-eGFP* to label different HSC populations demonstrated that Vwf^+ platelet/myeloid-biased HSCs are associated with megakaryocytes, whereas Vwf^- lymphoid/unbiased HSCs 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.

HSCs change location as niches are remodeled during aging

A growing body of evidence has indicated that HSCs redistribute within the BM upon aging. For instance, aged HSCs locate away from the bone surface (endosteum), compared with young HSCs, upon BM transplantation (36). This abnormal homing behavior correlates with increased BM HSC numbers and enhanced HSC egress into circulation (37). Recent studies using whole-mount immunofluorescence staining of murine long bones further reveal that aged HSCs are more distant to the endosteum, arterioles, Nestin-GFP^{high} cells and megakaryocytes, but HSC distance to sinusoids and Nestin-GFP^{low} cells appears unchanged, compared with young HSCs (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 BM mesenchymal stromal cells (BMSCs)

Studies regarding the absolute number of BMSCs during aging are controversial, with some suggesting an overall increase (41, 42), while others suggest unchanged (43, 44) or reduced numbers (45). It is noteworthy that BMSCs are heterogeneous, and the heterogeneity in the markers used to immunophenotypically define BMSCs might explain some of these discrepancies. Using *Nestin*-GFP to label murine BMSCs (46), different studies have reported reduced endosteal Nestin-GFP$^+$ cells in the aged BM (39, 40), which is consistent with reduced numbers of arteriolar αSMA$^+$, PDGFRβ$^+$ and NG2$^+$ cells (38). The age-related reduction of endosteal HSC niches might initiate lymphoid deficiency, since the endosteal microenvironment is reportedly important for lymphoid differentiation (29, 47-49). However, this notion has been refined more recently after elucidating dynamic interactions between B cell progenitors and perivascular BMSCs, which provide key signals for B lymphopoiesis (such as Cxcl12 and Il7), both in endosteal and central BM niches (50-53). Functionally, old BMSCs exhibit reduced colony-forming unit-fibroblast (CFU-F) capacity *in vitro* and reduced

expression of HSC niche factors (38). In this regard, revitalizing BMSCs to restore HSC-niche factors has been proposed as a strategy to prevent DNA damage in cultured HSCs (54).

BMSCs exhibit reduced osteogenesis with age, which is associated with lower osteopontin (OPN) secretion to the extracellular matrix (55). OPN negatively regulates HSC proliferation (56-58), and its decline might accelerate HSC divisions during aging. Supporting this idea, treatment with thrombin-cleaved OPN partially reverses age-associated phenotype of HSCs (55).

CC-chemokine ligand 5 (CCL5), a pro-inflammatory cytokine involved in bone remodeling (59), is reportedly increased with age. Researchers also found 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 BMSCs show adipocyte skewing (60). Adipocytes constitute a BM niche component that negatively regulates HSCs under steady-state (61) but promotes HSC regeneration after irradiation, although adipocytes appear overall dispensable for normal hematopoiesis (62). However, altered functions of adipose tissue, including insulin resistance and increased inflammation, have been described during aging (63) and MPN (64). Accumulation of BM adipocytes upon aging not only reduces hematopoietic reconstitution, but also disrupts bone fracture

repair (65). The later likely contributes to the increased risk of osteoporosis and bone fracture in the elderly population (66, 67).

BM aging is also associated with senescence of BMSCs, evidenced by increased p53/p21-mediated DNA damage, upregulation of p16(INK4a) and elevated levels of reactive oxygen species (ROS) (68-70). An age-dependent shortening of telomere has been found in telomerase-deficient (*Terc*$^{-/-}$) BMSCs; consequently, lethally-irradiated *Terc*$^{-/-}$ mice carrying wild-type BM cells display accelerated myelopoiesis (71). More recently, proteome analyses of human BM have unraveled nitric oxide (NO) synthesis and the urea cycle pathways as potential mediators for the crosstalk between old BMSCs and HSCs (72). Murine BMSCs show comparatively higher mRNA expression of neuronal nitric oxide synthase (nNOS, encoded by *Nos1* gene), as compared with other NOS isoforms, and *Nos1*$^{-/-}$ mice develop certain premature aging features, such as remodeled BM vasculature and myeloid skewing in peripheral blood (39). Given the importance of NO in vascular biology and balanced inflammatory responses, it is likely that NO pathways participate in the aged vascular remodeling and myeloid expansion partly by modulating the inflammatory response.

Remodeling of BM vasculature and endothelial cell (EC) functions

During aging, remodeling of BM endothelial vasculature is notable. Studies using

whole-mount confocal imaging, two photon intravital microscopy and flow cytometry analysis demonstrated an overall increased vascular density in aged mice (38, 73). Yet, distinct vascular beds show different, or even opposite alterations with age. Arterioles appear to be decreased, while sinusoids seem unchanged upon aging (39). Consistent with these observations, arteriole segments covered by Nestin-GFP^{bright} cells appear reduced (38). Transitional zone vessels (TZVs) containing type-H endomucin (EMNC)-high endothelial cells (which are enriched in the murine trabecular BM, where they support developmental bone growth (74)) are reduced in old mice (39, 73). In contrast, small capillaries (CD31^{high}EMCN^{-} cells with <6 μm diameter) are notoriously expanded in the central BM (39).

The function of vascular endothelium declines with age, as manifested by increased vascular leakiness, altered NO (possibly leading to vasodilation), increased ROS levels and decreased angiogenic potential (75). Poulos et al. reported that HSCs purified from young mice and cocultured with endothelial cells from old mice lack long-term multilineage hematopoietic reconstitution, whilst old HSCs cocultured with young endothelial cells maintain their self-renewal ability (75). Moreover, infusion of young ECs into aged, conditioned mice partly rejuvenates the old hematopoietic system (75). Kusumbe et al. identified high Notch activity in type-H endothelial cells and their associated subendothelial/perivascular cells (73), suggesting that reduction of endosteal

vessels upon aging is associated with impaired Notch signaling. Overexpression of the Notch ligand Dll4 in vascular endothelial cells can prevent myeloid skewing of hematopoietic progenitors (76) but cannot completely rescue HSC aging (73), which is consistent with another study that did not find altered Dll4 expression in the aged murine BM (40). A common finding across all these studies is the reduced endosteal activity of Notch ligands, manifested for instance by reduced expression of Jagged2 (Jag2) 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 HSCs near the sinusoids. Therefore, whereas the specific role of Dll4 during aging is not no clear, alterations of Notch signaling seem clearly 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 HSCs (since HSCs 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 (77). Several lines of evidence have indicated that these inflammatory cytokines drive myeloid/megakaryocytic differentiation. In aged-

related myeloid malignancies, such as myeloid proliferative neoplasms (MPNs) and chronic myelogenous leukemia (CML), serum IL-1β and IL-6 levels are elevated (78, 79). Pietras et al. reported that chronic IL-1 exposure induces HSC myeloid skewing at the expense of self-renewal (80). IL-1α/β regulates thrombopoiesis *in vitro* (81, 82), possibly explaining high platelet counts in aged mice (83). Defective phagocytosis of macrophages during aging induces expansion of platelet-biased HSCs through Il-1β signaling (84). Il-6 promotes thrombopoiesis either through a direct effect on BM megakaryocyte progenitors (39) or indirectly, by upregulating hepatic TPO levels (85). Yamashita et al. demonstrated that transient stimulation of TNFα prevents HSCs from necroptosis, and proposed that constitutive activation might lead to hyperproliferation of HSCs and exacerbated myelopoiesis in aging and myeloproliferative disorders (86). TGF-β produced by megakaryocytes regulates HSC quiescence, while megakaryocyte-derived TGF-β also stimulates TPO synthesis by BM stromal cells to enhance megakaryopoiesis (87). An elegant study by Haas et al. reported that acute inflammation induces proliferation of a stem-cell-like megakaryocyte progenitor to quickly replenish platelet loss in a process that requires IFN signaling (88).

Despite the well-known lymphocyte deficiency associated with aging, only the frequency and function (but not the absolute number of lymphoid-biased/balanced HSCs) appear to decline with age (89). In fact, during aging both platelet/myeloid-

biased and lymphoid-biased/balanced HSCs expand, but all these HSCs exhibit altered gene expression programs and myeloid/platelet skewing (83). These findings suggest a cell fate change of HSCs upon aging, with the net outcome being reduced lymphoid potential of HSCs and increased platelet/myeloid cell production. Two possible non-mutually exclusive explanations are: 1) Different HSCs suffer the same intrinsic abnormalities upon aging and 2) microenvironmental alterations specifically influence HSCs and their progeny. Supporting the second, distinct HSC subpopulations respond differently to inflammatory challenges during aging (90). Moreover, old lymphoid-biased/balanced HSCs seem to retain normal lymphoid differentiation potential when removed from the old microenvironment (5). Exogenous addition of IL-1 can block lymphocyte differentiation from old lymphoid-biased HSCs, 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-bias HSCs *in vitro* (84). IL-1 blockade can also attenuate myeloid expansion and inflammatory arthritis associated with the elderly (91).

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 (92). Therefore, exacerbated myelopoiesis during aging might induce myeloid/megakaryocytic HSC skewing through inflammatory

remodeling of the BM microenvironment. Many different cytokines that could directly bind to receptors on HSCs 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 inflammatory cytokines in the regulation of HSCs during aging.

Neuronal regulation by sympathetic adrenergic signaling

It has been reported that BM sympathetic stimulation of β_2- or β_3-adrenergic receptors (ARs) regulates HSC egress and G-CSF-induced mobilization (93-95). A recent publication has suggested that noradrenergic nerve fibers are reduced in the old murine BM (38). This study also indicates that surgical denervation of young BM induces premature aging of the hematopoietic system, although the inflammation caused by the surgical denervation might have certain influence. Similar reduction of nerve fibers has been reported in a mouse model of age-related blood disorder MPNs (78), suggesting that BM neuropathy might be a predisposition factor for normal or pathological myeloid expansion with age. However, another study has not found such reduction of BM noradrenergic fibers in aged mice (96). Moreover, whole-mount imaging and 3D 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

increased concentration of noradrenaline in the human plasma with age (97-100). Increased sympathetic activity causes myeloid-skewing of old HSC through activating β_2-AR, since exacerbated thrombopoiesis is present in old wild-type mice but is absent in old mice lacking β_2-AR or β_2- and β_3-ARs (39). A previous study reported that α-ARs directly regulate megakaryocyte migration, adhesion and proplatelet formation under stress, whereas α-ARs do not affect the earlier commitment of progenitor cells to the megakaryocyte lineage (101). Therefore, age-dependent increase in sympathetic innervation might activate different ARs as hematopoietic cells differentiate along the megakaryocyte lineage. Additionally, the sympathetic nervous system has been known to control inflammation in a context-dependent manner and the concentration of catecholamines and the expressions of different ARs can influence the inflammatory state of innate immune cells (102). Therefore, the increased sympathetic activity during aging might contribute to cytokine storm by activating inflammatory cells, and subsequently affect HSC differentiation.

Interestingly, β_3-AR exhibits opposite effects on lympho-myeloid skewing, compared with β_2-AR. Adult mice lackong β_3-AR show decreased frequency of endosteal lymphoid-biased HSCs and/or lymphoid multipotent progenitors (38, 39). Age-related remodeling of vasculature, such as reduced TZVs 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 has suggested that administration of a β_3-AR agonist in old mice rejuvenates most features of HSC aging (38). The same β_3-AR agonist can reduce myeloid expansion in a mouse model of MPN (78) and a murine model of Hutchinson-Gilford progeria syndrome (39). However, hematopoietic rejuvenation has not been detected in the peripheral blood of mice treated with this β_3-AR agonist over 40 weeks in another study (78). Likewise, elderly individuals with MPN who received β_3-AR agonist over 24 weeks did not show symptoms of rejuvenation in their peripheral blood counts in a human study (103). Five-month-old mice lacking β_3-AR reportedly show normal peripheral blood counts (39) or premature, lymphoid deficiency and myeloid skewing (38). Overall, the discrepancies between these studies suggests that modulating a single neuronal pathway might not be sufficient to rejuvenate hematopoiesis. This is maybe due to the multiple compensatory/adjustment mechanisms of the autonomic nervous system revealed, for instance, in the cardiovascular system (104). It is worth to mention that myeloid and lymphoid cells are a source of catecholamines (105). Adrenal-gland-derived adrenaline and immune-cell-derived (nor)adrenaline might contribute to increased levels of adrenaline and noradrenaline in the circulation of aged individuals (97-100) which, together with the increased BM noradrenergic innervation, might altogether activate ARs expressed in

BM cells. We propose that sympathetic regulation of lympho-myeloid skewing pivots on the activation or inactivation of different ARs. 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 (39). Further investigation of the underlying signaling pathways influencing hematopoietic differentiation during aging is warranted.

Other players in the BM microenvironment

Emerging data suggest that the progeny of HSCs can feed back to regulate their activity under homeostasis, raising the possibility that gerontological alterations in mature hematopoietic cells contribute to HSC aging. For instance, clearance of senescent CD62L^{low}CXCR4^{high} neutrophils by macrophages has been reported to modulate HSC niches (106). Frisch et al. found that aged macrophages are unable to engulf senescent neutrophils, leading to expansion of megakaryocytic-biased HSCs through IL-1β signaling (84). Another key player is the megakaryocyte, which can regulate HSC proliferation/quiescence through PF4, TPO and TGF-β (32-34). In the murine BM, around 20% of HSCs are spatially associated with megakaryocytes (32), and depletion of megakaryocytes expands platelet-biased HSCs (35). During aging, megakaryocytes increase in numbers, lodge closer to sinusoids, and abundantly form pseudopodial

extensions (proplatelets) (39). Of note, whereas both HSCs and megakaryocytes increase during physiological/premature aging, the distance between HSCs and megakaryocytes substantially increases (38, 39). The increased distance might compromise their interactions and could potentially promote HSC proliferation in the absence of inhibitory signals from megakaryocytes. However, whether age-related alterations of megakaryocytes can cause HSC aging requires further investigation.

Premature aging in Hutchinson-Gilford Progeria Syndrome (HGPS)

In HGPS, aberrant splicing of LMNA gene (encoding lamin A and C) leads to nuclear assembly of the truncated protein prelamin A (progerin) (107, 108). Certain hallmarks of murine hematopoietic aging, such as increased platelet counts, have been observed in HGPS (109). Given that normally-aged cells also exhibit increased levels of progerin (110), it is possible that normal physiological conditions and progeria might share some aging mechanisms. Grigoryan et al. recently reported that HSCs deficient in Lmna display a premature aging-like phenotype (111), suggesting a prominent role of laminA/C in HSC aging. The strong impact of progeria on growth and sexual maturation might be paralleled with altered endocrine regulation of HSCs, since growth hormones and sex hormones regulate HSC survival, proliferation and lineage commitment (112-115). However, it remains unknown whether premature hematopoietic aging in HGPS is a consequence of progerin accumulation in HSCs,

other hematopoietic cells and/or the microenvironment. In this regard, a mouse model carrying the human HGPS mutation (*Lmna*$^{G609G/G609G}$) (116) exhibits premature hematopoietic aging features; however, this premature hematopoietic aging is not observed in WT recipients carrying *Lmna*$^{G609G/G609G}$ hematopoietic cells (39). Microenvironmental alterations are observed in *Lmna*$^{G609G/G609G}$ mice, some of which are shared with normally aged mice, such as elevated pro-inflammatory cytokines (IL3, IL6, IL1, IFNγ), increased megakaryocytes with proplatelet-like extensions and increased megakaryocytes apposition to BM sinusoids. Of note, β_3-AR agonist improves the exacerbated myeloid expansion of progeroid mice correlated with restored apposition of HSCs to megakaryocytes. These results suggest that premature hematopoietic aging in HGPS is not HSC cell-autonomous but requires the remodeling of the microenvironment, which can be targeted to improve premature hematopoietic aging (39).

Aged HSCs/microenvironment: chicken or egg?

One standing key question relates to the order of events: do microenvironmental alterations initiate HSC aging and/or do old HSCs remodel the niche in a way that alters hematopoiesis? It is noteworthy that HSC aging is characterized by multiple HSC-intrinsic and -extrinsic events, and that different aging features might arise chronologically. For instance, during murine aging, defective lymphopoiesis manifests

relatively early (at the age of 8 months, approximately) (117), whereas increased platelet counts are not pronounced until 18 months of age (39). BM noradrenergic nerve fibers appear to be relatively decreased in adulthood (8 month-old adult mice, compared with 2 month-old young mice; Supplementary Fig. 5b of (38)), but these fibers appear increased in aged (20 month-old) mice (39). Lack of β_3-AR accelerates the loss of endosteal lymphoid-biased HSCs in 4-month-old mice, but it does not aggravate HSC aging in old mice, when β_3-AR signaling is already strongly reduced (39). In contrast, deficient 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 point towards a very active role of β_2-AR in aged hematopoiesis. Altogether, these evidences 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 lack of β_3-AR activity in the endosteal BM compromises this HSC niche and possibly lymphopoiesis, facilitating the relocation of endosteal HSCs to the central BM, where myelopoiesis is favored. Adrenergic switch from β_3-AR to β_2-AR could feedback to worsen the reduction of endosteal niches, since activation of β_2-AR on osteoblasts is known to restrain bone formation (118-120). However, the specific cell types involved in this regulation remain to be identified. It is possible that cells highly expressing β_2-AR replace those with high expression of β_3-AR over time, and/or

that BM niche cells ubiquitously increase β_2-AR expression while β_3-AR expression declines upon aging. These and other hypotheses require to be validated in future studies since the experiments were performed using global knockout mice (121). Future engineered models allowing tissue-specific deletion of β_2-AR and β_3-AR could provide further insights into the possible switch of β-adrenergic signaling during aging. Importantly, β_2-AR and β_3-AR have different affinities for norepinephrine and epinephrine; whereas β_3-AR shows higher affinity for norepinephrine over epinephrine, the opposite is true for β_2-AR (122). Another possibility is that BM concentrations of both neurotransmitters differ in aging, leading to imbalanced stimulation of the two receptors. The observation that old HSCs home in the BM away from endosteal regions suggests that HSC-driven niche remodeling mainly occurs in the central BM. 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 (84). Increased myeloid cells might provide an additional source of catecholamines in a feed-forward loop that results in megakaryocyte differentiation through sustained β_2-AR activation. We propose that accumulation of old HSCs causes microenvironmental remodeling by reinforcing β_2-adrenergic activity, expansion of central BM niches and enhanced myeloid/megakaryocyte differentiation. As a secondary outcome, alteration of

megakaryocyte localization (increased apposition to sinusoids correlating with increased megakaryocyte numbers and proplatelet formation) reducing megakaryocyte-HSC interactions might contribute to release HSCs from their quiescence state and further promote HSC proliferation with age.

Niche alterations might predispose to hematological neoplasms

The risk of developing myeloid malignancies significantly increases in individuals harboring clonal-hematopoiesis-related somatic mutations (123-128). In fact, some of these mutations are oncogenic drivers of myeloid malignancies (reviewed in (126)). However, the factors limiting the clonal expansion or allowing instead the mutant clones become dominant and, in some cases, cause disease, remain unclear. Interestingly, some niche alterations are shared between aging and age-related myeloid disorders, suggesting that niche remodeling could potentially favor the expansion of certain malignant clones. For instance, damaged neuro-MSC circuit promotes the development of cytokine storm created by the mutant HSCs, aggravating MPN progression (78). In myelodysplastic syndromes (MDS), abnormal production of cytokines from the microenvironment, dysfunction of BMSCs and osteolineage cells and vascular remodeling have been associated with disease initiation and progression (129, 130). Therefore, targeting the abnormal microenvironment could be a promising adjuvant therapeutic approach to treat hematological cancer in the future.

Conclusive remarks

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

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Players	Size	Functions/Mechanisms
Mesenchymal lineages	■ Decreased endosteal Nestin-GFP$^+$ cells ■ Increased non-endosteal Nestin-GFP$^+$ cells ■ Decreased αSMA$^+$ cells, NG2$^+$ cells, PDGFβ$^+$ cells	➢ Increased adipogenesis ➢ Decreased osteogenic differentiation ➢ Cellular senescence ➢ Altered nitric oxide (NO), 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, TNFα, INFγ, TGFβ	➢ IL-1β regulating HSC myeloid-skewing ➢ IL-6 regulating megakaryocyte differentiation ➢ TNFα protecting HSC from necroptosis ➢ TGF-β regulating megakaryopoiesis ➢ IFN regulating megakaryocytic bias
Sympathetic nervous system	■ Increased Th$^+$ nerve fibers	➢ β2-AR activation regulating HSC myeloid-skewing toward platelet production ➢ β3-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

Table 1. Microenvironmental players contributing to HSC aging

Figure 1

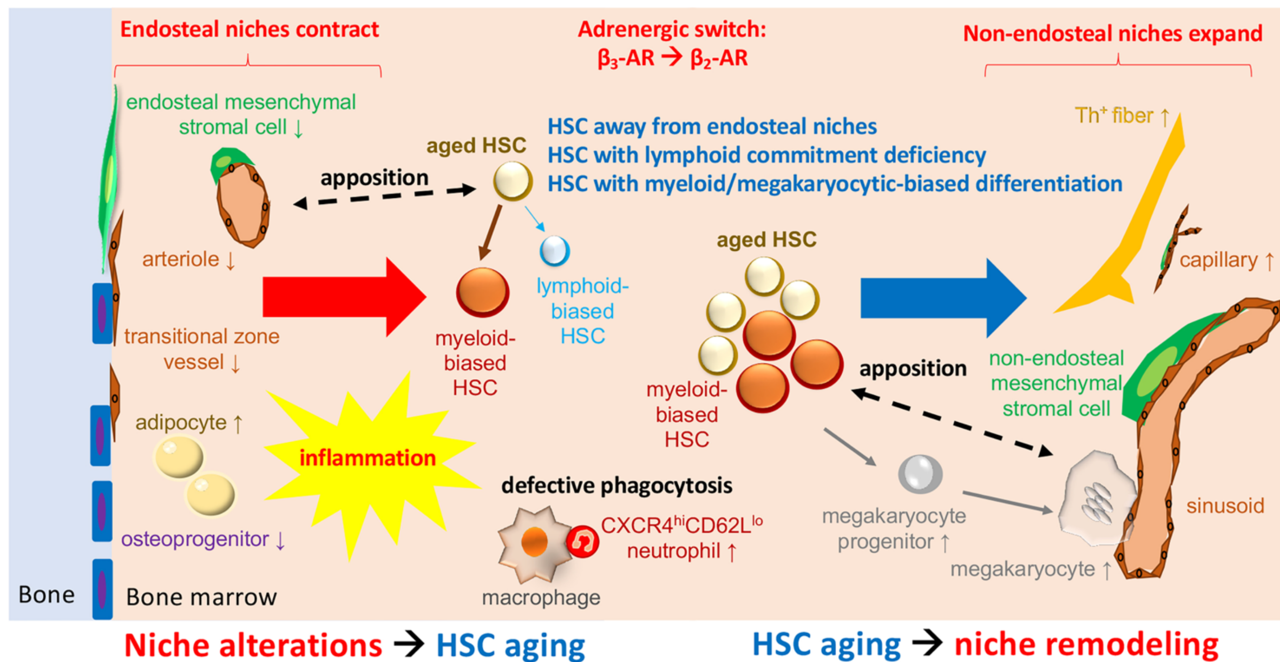

Figure 1. Schematic model of BM microenvironmental contributions to aging of the hematopoietic system. Loss of β_3-AR activity reduces endosteal niches, pushes HSCs away from the endosteum and favors myeloid bias at the expense of lymphopoiesis. Accumulation of aged HSCs in the central BM and increased β_2-AR activity causes expansion of central capillaries, myeloid cells and megakaryocytes, which locate further from HSCs.