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Hematopoietic stem cell niche maintenance during homeostasis and regeneration

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Abstract

The bone marrow niche has mystified scientists for many years, leading to widespread investigation to shed light into its molecular and cellular composition. Considerable efforts have been devoted toward uncovering the regulatory mechanisms of hematopoietic stem cell (HSC) niche maintenance. Recent advances in imaging and genetic manipulation of mouse models have allowed the identification of distinct vascular niches that have been shown to orchestrate the balance between quiescence, proliferation and regeneration of the bone marrow after injury. Here we highlight the recently discovered intrinsic mechanisms, microenvironmental interactions and communication with surrounding cells involved in HSC regulation, during homeostasis and in regeneration after injury and discuss their implications for regenerative therapy.

Hematopoiesis is a continuous process of blood-cell production occurring through the orchestrated proliferation, self renewal and differentiation of HSCs in the bone marrow followed by egress of mature progeny into the circulating blood1–4. HSCs are the only cells capable of producing all blood cell lineages throughout life5. Within the bone marrow exists a tightly controlled local microenvironment, or niche, that regulates the quiescence, proliferation and differentiation of HSCs6. Regulatory signals within the niche emanate from surrounding cells in the form of bound or secreted molecules and also from physical cues such as oxygen tension, shear stress, contractile forces and temperature4,7,8. During homeostasis, the majority of HSCs are quiescent9 but can become activated to proliferate and differentiate in response to infectious stress such as interferonmediated signaling10–12.

High-dose chemotherapy or radiation treatment for hematological malignancies such as leukemia, lymphoma or multiple myeloma may induce damage to the bone marrow microenvironment and limit the regeneration and differentiation potential of HSCs by reducing their numbers and causing functional deficits among the remaining HSCs1,13–15. In the 1960s, the promise of stem cell therapy was ignited after Dr. E. Donnall Thomas performed the first successful allogeneic bone marrow transplant, which was a groundbreaking procedure that eventually revolutionized patient care and led to substantial clinical advances in cancer treatment (Box 1). Difficulty in finding suitable adult allogeneic donors along with low stem cell yield from cord blood donation has led to the investigation of stem cell expansion methods (Box 2). However, HSC expansion has been very difficult to achieve because of an inability to maintain these cells in synthetic culture environments that differ from the native bone marrow microenvironment. A promising alternative approach would be to reprogram somatic cells directly into HSCs16–18. However, most transcription factors conferring HSC identity have also been associated with leukemia, raising the potential for malignant transformation using this approach. The success of this cell therapy

also relies on the ability of HSCs to engraft, self renew and differentiate into multiple blood cell lineages5. Uncovering safe techniques to promote HSC expansion in vivo without inducing cancerous transformation (Box 3), along with cellular and environmental factors that encourage HSC lodgment while maintaining stemness, could form the basis for new therapeutics and in turn result in expedited regeneration with improved clinical outcomes.

The different cell types of the HSC niche that are essential in HSC maintenance and regeneration are discussed in this Review, along with key regulators of survival and self renewal through intrinsic and extrinsic mechanisms during homeostasis, stress and aging. We propose future directions with promise for advancement in the field and discuss the therapeutic implications of the new players that seem to orchestrate the process of HSC niche regeneration in the bone marrow.

Cellular players of the HSC niche

Technological advances in bone marrow imaging unveiling cellular localization specificities, combined with conditional deletion of crucial regulatory factors from candidate cell types in mouse models, have uncovered several candidates involved in HSC maintenance (Fig. 1)19,20. The bone marrow ‘stroma’ can initiate and maintain hematopoiesis, as demonstrated by the reconstitution of a hematopoietic microenvironment within an ossicle model in which stromal cells are seeded onto a transplanted biomaterial scaffold19,20. Much progress has been made in characterizing the cellular composition of the niche.

Perivascular cells in the HSC niche

Of the cell types that have been suggested to regulate HSC maintenance and regeneration, cells of the vasculature have been the focus of considerable interest. Human bone marrow analyses have suggested that perivascular cells expressing the melanoma-associated cell adhesion molecule (MCAM, also called CD146) are stromal progenitors in the bone marrow21 (Fig. 1). A subset of CD146 cells in humans, and a large fraction of perivascular stromal cells in mice, express platelet-derived growth factor receptor-α (PDGFR-α), CD51 (also called ITGAV) and the intermediate filament protein nestin22,23. These cells account for all bone marrow mesenchymal stem and progenitor cells (MSPCs) as measured by colony-forming–unit fibroblastic activity assay22. In addition to being localized near both HSCs and adrenergic nerve fibers, these cells express high levels of genes mediating HSC maintenance and retention, including those encoding the cytokines chemokine (C-X-C motif) ligand 12 (CXCL12) and stem cell factor (SCF)22.

Perivascular stromal cells expressing high amounts of CXCL12, known as CXCL12-abundant reticular (CAR) cells, regulate HSC self renewal, proliferation and trafficking24. Although the exact composition of CAR cells remains unclear, they comprise cells marked by nestin, myxovirus resistance-1 (Mx-1), leptin receptor (Lepr), the transcription factor paired related homeobox-1 (Prx-1) that marks cells of the limb bud mesoderm and the transcription factor osterix (Osx, also called SP7) that is necessary for osteoblast differentiation (as described further below)23,25–27. Stromal cells labeled by Mx1-Cre are also enriched for MSPC activity in culture and label the osteolineage27. Lepr on

perivascular cells is the receptor for the adipocyte-secreted hormone leptin that promotes energy metabolism28. These Lepr+ cells have an MSPC phenotype and express SCF and CXCL12 (refs. 26,29). In addition, perivascular Lepr+ cells largely overlap with stromal cells marked by nestin-GFP22,30 and appear to label a self-renewing mesenchymal population that contributes to adipocyte and bone regeneration31,32.

Endothelial cells in the HSC niche

Endothelial cells line the surface of blood vessels, bridge blood and tissues in the bone marrow and promote HSC maintenance and regeneration after injury33,34. Endothelial cells are ensheathed with pericytes or adventitial reticular cells, including nestin+ CAR cells35 (Fig. 1). Studies in embryoid bodies have suggested that endothelial cells and hematopoietic cells derive from a common multipotential precursor cell, the hemangioblast36,37, but there is no evidence in the developing bone marrow that endothelial cells can give rise to pericytes38.

Studies in mice originally defining HSCs by a combination of signaling lymphocytic activation molecule (SLAM)-family surface receptors revealed a preference for HSCs to associate with sinusoidal endothelium in the bone marrow, suggesting a potential regulatory role for the endothelium39. Recent reports have confirmed that bone marrow endothelial cells are able to support hematopoiesis through the expression of essential surface makers, including E-selectin (also called SELE)36, and upregulation of ‘angiocrine’ factors such as fibroblast growth factor 2 (FGF2), delta-like 1 (DLL1), insulin-like growth factor-binding protein 2 (IGFBP2), angiopoietin 1 (ANGPT1), desert hedgehog (DHH) and epidermal growth factor (EGF)34,37,40–42.

Osteoblasts and cell identification challenges

Perhaps the most controversial cellular components of the bone marrow niche are the bone-forming osteoblasts, as initial studies pointed toward their regulatory role in hematopoietic maintenance43,44. However, more recent studies in which CXCL12 or SCF—cytokines that are critical for HSC regulation in mice—were conditionally depleted from mature osteoblasts (marked by osteocalcin (Bglap)-Cre or Col2.3-Cre) showed normal cellularity and lineage composition in the bone marrow and spleen, normal blood counts and a preserved ability to reconstitute in irradiated mice25,26,29, suggesting that osteoblasts do not directly maintain HSCs in the bone marrow.

A stumbling block in the field has been a lack of specificity of the genetic promoters that mark subsets of mesenchymal lineage cells28. For example, although the expression of the osteoblast marker Osx is restricted in short-lived osteoblast progenitors in the adult bone marrow, it is also expressed perinatally in immature MSPCs that are long lived in the adult bone marrow31,45. The ability to mark stromal cells with increased precision and differentiate between those present during development and those in postnatal tissues will clarify the cells responsible for promoting HSC maintenance.

Sympathetic nerves

It has been shown that trafficking of HSCs into the bloodstream during steady state is tightly regulated through the circadian release of adrenergic signals from the sympathetic nerves in the bone marrow46, suggesting that sympathetic nerves might regulate HSC function. Nestin-expressing MSPCs touch sympathetic nerves directly in the bone marrow and express high levels of HSC maintenance genes during steady state. Electromechanical coupling between noradrenergic nerves and nestin-expressing cells through their β3-adrenergic receptor leads to downregulation of HSC maintenance genes as CXCL12, Angpt1, Kit ligand (Kitl) and vascular cell adhesion molecule 1 (Vcam-1) followed by HSCs egress from the bone marrow23,47. Nonmyelinating Schwann cells, which are wrapped around sympathetic nerves, have also been shown to localize close to HSCs and maintain HSC quiescence by activating transforming growth factor-β (TGF-β)-SMAD signaling48.

Macrophages

Macrophages have been added to the pool of key nicheregulating cells given their effect—through an unidentified cytokine— on nestin-expressing niche cells to promote HSC retention by inducing CXCL12 secretion49,50. Macrophages have thus been shown to have a parallel and antagonistic role compared to sympathetic nerves49. Interestingly, granulocyte colony-simulating factor (G-CSF) treatment in mice (which promotes the mobilization of HSCs and production of granulocytes) depletes both macrophages and osteoblastic cells50,51 and activates sympathetic neurons to release norepinephrine in the bone marrow microenvironment52. As osteoblastic cells do not express the G-CSF receptor, this finding suggests that osteoblast suppression occurs indirectly, possibly through signaling in bone marrow macrophages50,51, and sympathetic activation47,52.

Dispensable HSC niche cell types and negative regulators

Select bone marrow cell types have been shown in genetic animal models to be either dispensable or negative regulators of the HSC niche. After chemotherapy or irradiation, adipocytes have been found in increased numbers in the bone marrow because of adipogenic differentiation of MSPCs53, where their increased presence can hamper hematopoietic recovery2 and can be used as a diagnostic indicator of bone marrow aplasia53. Bone-degrading osteoclasts have also been suggested to be dispensable for HSC maintenance in mouse models including op/op mice, which are deficient in a cytokine needed for osteoclast differentiation, and Fos–deficient and Rankl-deficient mice, which are deficient in osteoclasts54,55.

Bone marrow niche signals

The niche must preserve the properties of the stem cell while regulating stem cell maturation and differntiaion56,57. A complex milieu of components is responsible for HSC maintenance, including soluble mediators, intrinsic signaling pathways and microenvironmental signals, such as those mediated by adhesion molecules and local oxygen tension, as well as interactions with other cellular niche constituents.

Perivascular-derived SCF and CXCL12 in HSC maintenance

A conditional deletion approach to eliminate key factors from candidate niche cells in animal models has recently defined important regulators of HSC maintenance26. Targeted deletion studies have shown that SCF is expressed by both perivascular and endothelial cells26. These studies also suggested that nestin-expressing niche cells do not contribute to SCF secretion, which seems to contradict previous reports of nestin-expressing stromal cells as an important regulator of the HSC niche23; this discrepancy is likely due to varying nestin-driven transgenic Cre expression and recombination levels among perivascular cells in the bone marrow26. Indeed, perivascular cells expressing Lepr and nestin+ reticular cells localize largely together in the bone marrow, but because of the heterogeneity in genetic labeling of perivascular cells, the contribution of this population of cells in SCF secretion will require further analyses22,30. However, Scf expression has not been found in bone-lining osteoblast lineage cells marked by Col2.3-Cre in either the diaphysis or trabecular bone, indicating that osteoblasts are not an essential source of SCF for HSC maintenance26.

CXCL12 is a niche factor that has been shown to regulate HSC functions such as retention in the bone marrow, quiescence and the ability to induce multilineage reconstitution25,29,58. Initial studies demonstrated that deletion of the CXCL12 receptor, CXCR4, in Mx1-Cre mice resulted in substantial reductions in HSC (defined in this study as CD34−c-Kit+Sca-1+Lin−) numbers in the bone marrow because of an enhanced exit from quiescence24. Deletion of CXCL12 from CAR cells using Osx-Cre markedly reduced CXCL12 expression but showed no effect on HSC maintenance and led to mobilization of HSCs to the blood25. Similar results were observed with Lepr-Cre deletion29, suggesting that reticular cells around sinusoids are crucial for HSCs in bone marrow.

In contrast, the Prx1-Cre deletion strain in which CXCL12 is deleted more broadly in the appendicular skeleton revealed a role for CXCL12 expressed by these stromal cells in maintaining HSC repopulating activity and quiescence25,29. Given the recently reported role of arterioles in regulating HSC quiescence and the high levels of CXCL12 expression in periarteriolar nestin-GFP+ cells30, further studies will be needed to define the stromal subsets forming HSC quiescent niches. However, deletion of CXCL12 with nestin-Cre has not revealed any defect in HSC frequencies, suggesting that recombination did not occur in nestin-GFP+ cells even though these cells are probably a major contributor of CXCL12 content in bone marrow. By contrast, deletion of CXCL12 in endothelial cells using Tie2-Cre revealed minimal defects in HSC numbers and competitive reconstitution abilities25,29.

Conditional deletion of CXCL12 in osteoblasts (Col2.3-Cre or Bglap-Cre) did not result in any HSC defects15,33. However, deletion with Col2.3-Cre resulted in reduced levels of B and T cell reconstitution and depletion of early lymphoid progenitors in the bone marrow29. These results argue that the mesenchymal cells committed to the osteoblast lineage are dispensable for HSC maintenance but may regulate lymphoid progenitors.

Notch and HSC maintenance

The Notch pathway plays an important part in many developmental processes, and it has been suggested to regulate many adult stem cell fate decisions (reviewed in refs. 59,60).

Although Notch signaling has been found to regulate HSC recovery after stress in mice, it remains under debate whether canonical Notch signaling contributes to HSC maintenance or whether this signaling pathway is dispensable37,60–63.

Expression of Notch receptors early in hematopoiesis may be involved in cell differentiation decisions and may be used to identify specific progenitor cell types with a predetermined cell fate61: Notch1 was found to promote T cell commitment and has been shown to specify megakaryocyte fate, whereas Notch2 marked primarily erythroid progenitor cells61,62,64. Notch1 and Notch2 receptors have also been found to promote the expansion of long-term repopulating HSCs (LT-HSCs; CD34−FLT3−Lin−c-Kit+Sca-1+) while preserving self-renewal ability34,37. Short-term repopulating HSCs (ST-HSCs; Lin−CD34+Sca-1+c-Kit+) secrete vascular endothelial growth factor A (VEGF-A) when exposed to soluble SCF in vitro, which in turn stimulates the translocation of the Notch ligand Jagged-2 (Jag2) to the endothelial cell surface37. Jagged-2 supports the expansion of STHSCs through expression of Notch1 and Notch2 receptors37. After conditional deletion of Jagged-1 in endothelial cells, there were reductions in the numbers and function of LT-HSCs during homeostasis and during hematopoietic regeneration after irradiation, suggesting that this Notch ligand is required to maintain the quiescence and self renewal of HSCs65.

Although these findings suggest that canonical Notch signaling promotes HSC self renewal and maintenance, other studies have suggested that it may also be dispensable63,65,66. Using a dominantnegative approach to inhibit Notch signaling in hematopoietic progenitors from mice, LT-HSCs were maintained after transplantation, suggesting that Notch signaling is dispensable63. Furthermore, the Notch ligand Dll4 on erythroblasts has been shown to promote premature T cell differentiation in HSCs unless this signal is suppressed by leukemia/lymphoma related factors that are also released by erythroblasts to promote self renewal67.

The difficulty in resolving opposing results regarding the role of Notch in hematopoiesis may lie in the innate complexity of the signaling pathway and methodologies used to evaluate its function. For example, inhibiting one protein required for the transcriptional activation of Notch signaling may result in the activation of compensatory pathways68. Resolution of the specificity of the cell types targeted for genetic deletion, the levels of Notch pathway inhibition and a study of the pathways under comparable conditions will be required to determine the extent to which Notch is necessary for HSC regulation.

A complex and unresolved role for Wnt in hematopoietic regulation

Wnt, similar to Notch, is another pathway that has been demonstrated to regulate the development of various tissues, including hematopoietic tissues (reviewed in ref. 69). Initial β-catenin (Ctnnb1, an important component of the Wnt pathway) gain-of-function studies investigating the role of canonical Wnt demonstrated that activation of β-catenin in HSCs led to HSC expansion in vitro while maintaining an immature HSC state and promoting trilineage reconstitution70,71. Conversely, an opposite result indicated that the constitutive activation of β-catenin using Mx1-Cre in mice led to induced cell cycle entry, impaired differentiation, exhaustion and reduced multilineage reconstitution after transplantation followed by death72,73. The different methods used to constitutively activate β-catenin in

the aforementioned studies potentially led to varying Wnt signaling levels in HSCs and might account for the observed discrepancies.

Loss-of-function studies in mice found that deleting the canonical Wnt ligand Wnt3a led to lower HSC and progenitor cell numbers in the fetal liver, decreased self renewal and reduced long-term repopulation ability74, supporting a role for canonical Wnt in regulating HSC self renewal, although the specific underlying mechanism has not yet been uncovered. Conditional deletion of β-catenin using Vav-Cre or specific overexpression of a negative regulator of canonical Wnt, Dkk1, in osteoblastic cells (Col2.3-Cre) in vivo also resulted in decreased hematopoietic reconstitution after transplantation and further confirmed the role of Wnt in HSC self renewal74–77. However, conditional deletion of both β-catenin and γ-catenin in Mx1-Cre mice did not affect self renewal or hematopoiesis after transplantation78,79. Despite deletion of both β-catenin and γ-catenin, Wnt signaling remained present, suggesting that a compensatory β-catenin homolog may exist79,80.

Although the majority of studies have investigated canonical Wnt, the noncanonical Wnt pathway has also been suggested to affect HSC behavior. The noncanonical Wnt ligand Wnt5a has been suggested to inhibit canonical Wnt signaling, inhibit cell proliferation in vitro and increase the repopulating ability of HSCs in a mouse model81 by acting through the receptor-like tyrosine kinase (Ryk) receptor82. LT-HSCs have been reported to express the members of noncanonical Wnt signaling flamingo (Fmi, also called Celsr) and frizzled 8 (Fzd8), which promote quiescence during homeostasis by preventing nuclear localization of nuclear factor of activated T cell (NFAT), suppressing interferon-γ (IFN-γ) expression and antagonizing canonical Wnt signaling83. Stress-mediated activation of HSCs in mice may result in the repression of noncanonical Wnt signaling and enhanced canonical Wnt signaling, leading to HSC activation83.

Generation of a gradient of canonical Wnt signaling levels confirmed the previously noted differences in HSC behavior, where HSCs favored low levels of canonical Wnt signaling, leading to the maintenance of an immature phenotype and enhanced long-term repopulation capacity as opposed to moderate and high levels of Wnt signaling, which impaired the ability of HSCs to repopulate84. Whereas complete loss of Wnt signaling resulted in impaired self renewal, low levels of Wnt signaling led to HSC maintenance, demonstrating a high sensitivity to dosage, which must be considered for potential clinical translation. Although the role of Wnt in HSC maintenance remains unresolved, conditional deletion of canonical and noncanonical Wnt regulators from key niche cells, such as perivascular stromal cells, could further clarify its role.

N-cadherin

Original reports of HSCs homing near N-cadherin (Cdh2)- expressing osteoblasts led to the idea that N-cadherin expression on HSCs is responsible for homophilic binding to N-cadherin– expressing osteoblasts43. There has been considerable effort spent on elucidating the role of this adhesion receptor in the HSC niche. Low as compared to intermediate levels of expression have been suggested to mark a more active or reserved state, respectively85, and overexpression of N-cadherin in hematopoietic stem progenitor cells (HSPCs) reduced their proliferation in vitro86. However, conditional deletion of N-cadherin in HSCs using

Mx1-Cre mice revealed normal HSC frequency and an unaffected ability to reconstitute irradiated mice with primary and secondary transplantations, suggesting that N-cadherin is not required to cell-autonomously maintain HSCs87. Other studies that have conditionally deleted N-cadherin in osteolineage cells have not found an HSC phenotype88,89. These studies thus suggest that N-cadherin is dispensable for HSC function, although it could potentially mark an HSC subset or be capable of modulating HSC function when its expression is enforced. Because N-cadherin is highly expressed on HSC niche cells23, it may regulate MSPC differentiation, as suggested by a reduction of mineralized bone when deleted in the osteolineage89.

TGF-b. Various sources of TGF-β have been linked to HSC maintenance in the bone marrow niche. Nonmyelinating Schwann cells that wrap nerves in the bone marrow can secrete TGF-β activator molecules into the niche to induce TGF-β–SMAD signaling in HSCs, which contributes to maintenance and self renewal through increased phosphorylation of Smad2 and Smad3, causing HSC dormancy48. TGF-β1 was also found to stimulate myeloid-biased HSCs to proliferate while inhibiting lymphoid-biased HSCs90. TGF-β blockade in mice revealed that TGF-β inhibition shortly after chemotherapy results in increased hematopoietic cycling and accelerated hematopoietic reconstitution, whereas inhibition during homeostasis did not induce HSPC cycling91, suggesting that blocking TGF-β signaling during regeneration could enhance hematopoietic recovery. Cripto (also called TGDF1), a protein that blocks TGF-β signaling, binds to the GRP78 (also called HSPA5) receptor on hypoxic HSCs and activates the PI3K-Akt pathway, which results in the maintenance of HSCs located in the ‘endosteal niche’. Blocking Cripto-GRP78 signaling with the N-20 blocking antibody led to mobilization of HSCs from the endosteal region to the central marrow area but did not change HSC frequency in the bone marrow, peripheral blood or spleen, indicating that local mobilization was induced but peripheral circulation was not92. Endosteal cells expressing Cripto on their cell surface included Alcam−Sca-1+ and, to a lesser extent, Alcam+Sca-1− cells92. Taken together, these niche-regulating soluble factors and signaling pathways implicate vascular niches as regulators of HSC self renewal and maintenance.

Other niche factors regulating HSC function

Additional secreted factors from the bone marrow microenvironment have been shown to regulate HSC maintenance in vivo. Tie2-expressing HSCs associate closely with angiopoietin 1–expressing stromal cells, and this interaction has been shown to enhance the adhesion of HSCs to osteoblastic cells through an upregulation of integrin β1, leading to HSC quiescence, stem cell renewal and protection from myelosuppressive stress93. Given the role of MSPCs in the HSC niche, it will be important to define which cells of the microenvironment secrete angiopoietin 1.

Pleiotrophin secretion by bone marrow sinusoidal endothelial cells regulates HSC maintenance through binding and inactivating phosphatase activity induced by the transmembrane protein tyrosine phosphatase receptor type Z (PTPRZ) and retention in the bone marrow in vivo through the CXCR4-CXCL12 axis41,94. Interestingly, the pleiotrophin contribution from various primary stromal cell lines prepared from the aorta-gonad-

mesonephros region of mouse embryos was also found to mediate hematopoietic regeneration94, suggesting that broad sources of pleiotrophin may yield HSC maintenance and regeneration and that their individual contributions should be further resolved. Plastic-adherent bone marrow stromal cells are able to secrete the retinaldehyde-inactivating enzyme CPY26 to sustain low levels of retinoic acid signaling that would otherwise mediate terminal differentiation and promote a primitive HSC phenotype and HSC function and self renewal, as assessed in vitro and in vivo95. Other cell types found in the bone marrow, such as endothelial cells and osteoblasts, also express CYP26, but their individual roles in maintaining low levels of retinoid acid signaling have not been confirmed95,96.

Location of the niche

With the discovery of specific HSC surface markers, improved histological imaging capabilities in both live mice and fixed tissues and conditional deletion of HSC regulatory molecules from specific niche cells, the original view that osteoblasts regulate HSC maintenance and differentiation in the bone marrow niche has been revised, and the relevant role of perivascular cells has been uncovered30,97. Several imaging studies have suggested that HSCs may lodge in specific areas of blood vessels, possibly because of endothelial adhesive molecules98. Homeostatic HSCs are homogenously distributed, whereas transplanted HSCs preferentially home to the trabecular bone region. These HSCs seem to have enhanced regenerative and self-renewal capacities compared with those that localize to the diaphysis region99.

The use of intravital microscopy imaging of the mouse calvarium recently revealed that after injection into nonirradiated mice, all HSCs and progenitor cells homed and localized very close (within 16 μm) to the vasculature100 (Fig. 2). After transplantation into irradiated mice, however, most HSCs were found closer (within 15 μm) to the endosteum, where they were able to generate all peripheral blood lineage cells100. A subsequent study using a fiber optic imaging system showed that transplanted LT-HSCs were able to localize nearby vascular structures within 5 h of transplantation, regulated in part by VCAM-1 expression on endothelial cells101. More recent image analyses using laser-scanning cytometry, which enabled quantitative imaging of fluorescently labeled cells within mouse tissue sections, revealed that primitive progenitors (Lin−CD48−CD41lo/−c-Kit+) were enriched in the endosteal region and were associated with blood vessels97.

High-resolution imaging in combination with genetic labeling approaches have also unveiled a structural compartment, called the hemosphere, located in the metaphyseal region near the growth plate consisting of endothelial, mesenchymal and hematopoietic cells, which has been suggested to promote rapid HSC proliferation and clonal expansion102. Whole-mount confocal immunofluorescence to image mouse bone marrow further defined the association between HSCs and the vascular structures, showing that quiescent HSCs (defined as Ki-67− or BrdU label–retaining CD150+CD48−Lin−CD41− cells) localized mainly with small arterioles of the endosteal region, which were ensheathed by NG2 (CSPG4)+ pericytes39. After HSC activation, however, these cells moved away from the NG2+ periarteriolar niche to the Lepr-expressing perisinusoidal niche30. Although most

proliferating HSCs are associated with sinusoids, the density of these vessels is such that the short distances were not statistically different from a random placement.

Although most studies used a 12-cell diameter or a distance of 100 μm from the bone to define the endosteum, three-dimensional imaging has shown that HSCs are concentrated in a much larger endosteal fraction30. About 80% of HSCs lie within 50% of the distance (on average ∼220 μm) to the bone surface, whereas only 20% are found within 50% of the distance toward the central vein (Fig. 2). It thus remains unclear how the bone influences this HSC distribution, as other confounding structures (for example, arterioles) are also concentrated in this area.

HSCs and stress

HSCs may be challenged by diverse sources of stress, including oxidation, anemia, hypoxia, radiation, cytotoxic chemotherapy and inflammation, which can disrupt homeostasis and impair regeneration103,104. Ionizing radiation and chemotherapy, which are commonly used to treat hematopoietic malignancies and leukemia, invariably lead to bone marrow injury and alteration in cell composition1. After chemotherapy there is a progression of blood cell death based on the innate lifespan of the cell, with granulocytes preceding platelets followed by erythrocytes105, and chronic effects in bone marrow cells that include reductions in the amounts of progenitor cells that have increased cycling105.

Irradiation

Effects on the bone marrow from irradiation resemble those induced by chemotherapy, including chronic toxicity that can affect the dynamics of bone marrow cell production, maturation, trafficking and lifespan105. Repeat exposure to radiation can lead to the development of cancer, weakened hematopoietic mobilization and delayed hematopoietic reconstitution, leading to impaired bone marrow regeneration after transplantation106. HSCs are sensitive to radiation and react by increasing apoptosis in a dose- and time-dependent manner, which can be attenuated by VEGF-induced expression of myeloid cell leukemia-1 (MCL1) in hematopoietic progenitor cells107,108. Administration of thrombomodulin or activated protein C (aPC) within 24 h after lethal irradiation in mice has been reported to have a radiomitigating effect and result in improved hematopoietic recovery109. Although the underlying cellular and molecular mechanisms remain to be fully uncovered, a subsequent study demonstrated that aPC can promote antiapoptosis through binding to the protein C receptor on HSCs110. Additionally, a group of small-molecule inhibitors of cyclin-dependent kinase 4 (CDK4) and CDK6 can also mitigate the hematopoietic toxicity induced by radiation by promoting pharmacological quiescence of early hematopoietic stem and progenitor cells in the bone marrow111. These options may present alternative avenues to mitigate the toxicities of irradiation.

The shift from survival to initiation of apoptosis after irradiation of HSCs is regulated by the B cell CLL/lymphoma 2 (BCL-2)-family proteins and p53 (refs. 112,113). The p53-interacting protein known as apoptotic stimulating protein of p53 (ASPP1 or PPP1R13B) is responsible for altering the transcriptional activity of p53 to promote apoptosis113. Chronic inflammation, a long-term effect of ionizing radiation, induces increased amounts of plasma

tumor necrosis factor-α (TNF-α), IFN-β, interleukin-6 (IL-6) and C-reactive protein, which can suppress the recovery of residual HSCs114. Regeneration therapies after radiation could therefore potentially benefit from treatments aimed at reducing inflammation.

Oxidiative stress

Oxidative stress is the result of an overabundance of cellular reactive oxygen species (ROS) accumulation formed by the partial reduction of oxygen or a defect in the antioxidant protection mechanism115,116. The ability of hematopoietic tissues to maintain redox status is crucial to maintaining normal hematopoiesis115, as free radicals and ROS produced by high doses of radiation alter HSC repopulating ability and damage the bone marrow vasculature13,117. Substantial delay in DNA double-strand break repair after irradiation leads to DNA damage113, which can be exacerbated by the DNA damage caused by increased HSC proliferation after radiation118. ROS can activate DNA damage response pathways mediated by p53, ATM, 53BP1 (TP53BP1), CHK2 and FOXO3a, which in turn activate the HSC cell cycle inhibitors p16INK4a, p14ARF and p21CIP1, promoting senescence and loss of stem cell function118. Therapeutic strategies aimed at reducing excessive ROS accumulation after radiation may also provide a path to expedite recovery.

Lessons from radioresistant cells

Although Lessons from radioresistant cells. Although the majority of HSCs are adversely affected by irradiation, radioresistant cell populations also exist in the bone marrow. For example, mature megakaryocytes localize near the trabecular surface after irradiation, where they produce growth factors that stimulate increased cycling of CD45− nestin-expressing MSCs, leading to their differentiation into preosteoblasts, potentially increasing hematopoietic stem cell number as well119. Numerous studies have indicated the effectiveness of various cytokines at stimulating radioresistant cell populations for promoting hematopoietic recovery in both animal models and humans120. In particular, administration of a single dose of SCF, FLT3 ligand, hrombopoietin (TPO) and IL-3 within 2 hours after irradiation effectively led to reduced cytopenia and improved hematopoietic recovery in mice and nonhuman primates and could potentially serve as a treatment method for patients after accidental or intentional radiation exposure121,122. Whether other niche-regulating stromal cells are affected by radiation stress remains unknown, but their identification could potentially uncover new target cell sources to increase bone marrow function in patients after irradiation.

Regeneration of the HSC pool after injury

Substantial efforts have been dedicated toward uncovering the mechanisms regulating HSC niche maintenance, yet the regenerative process that takes place after hematopoietic injury remains more elusive (Fig. 3). Various signaling pathways implicated in homeostasis have also been shown to be involved in regeneration and are mediated in part by the bone marrow vasculature.

Notch signaling

Notch signaling appears to be important for HSC regeneration, as it has been shown that angiogenic factors released by endothelial cells stimulate Notch ligands to prevent HSC exhaustion after myeloablation from lethal irradiation37. Activation of the Akt-mTOR pathway in endothelial cells also promotes hematopoietic stem and progenitor cell regeneration through regulation of angiocrine factors34. In addition, expression of the canonical Notch ligand Jagged-1 by endothelial cells also supports hematopoietic regeneration by balancing the levels of self renewal and differentiation to prevent premature HSC exhaustion65. In HSCs, Notch signaling activation enhances megakaryocyte production and platelet formation by interacting with Dll1 ligand expressed by OP9 stromal cells64, whereas Notch2 signaling through Jagged-1 enhances the generation of shortterm repopulating multipotent progenitor cells and long-term HSCs after myeloablation while hindering myeloid differentiation62.

Regulating apoptosis

A recent investigation further highlighted the regulatory effects of endothelial cells on HSC regeneration after radiation injury123. In mice, deletion of the proapoptotic genes Bak and Bax in Tie2-expressing HSCs and endothelial cells prevented their depletion after irradiation and resulted in radioprotection of HSCs123. Deletion of Bak and Bax in VE-cadherin–Cre mice, which only targets a small subset of HSCs, led to an increase in 15-day survival but resulted in no statistical difference in 30-day survival compared to VE-cadherin–Cre+ Bakflox/+; or Baxflox/+ and VE-cadherin–Cre− mice123. These results indicate that the hematopoietic response to radiation is mediated by HSC-autonomous effects as well as endothelial cell–mediated mechanisms123. In addition, these findings confirm previous studies showing that reducing radiation-induced apoptosis of HSCs through repression of the proapoptotic protein PUMA (BBC3) can promote HSC recovery40.

TGF-β

During regeneration after myelosuppression from chemotherapy, there is transient activation of the TGF-β pathway in HSCs91, and its blockade in this setting—but not during homeostasis—enhances hematopoietic reconstitution, hindering the ability of hematopoietic cells to fall back into a quiescent state91. Clinical use of TGF-β inhibitors could result in enhanced multilineage hematopoietic regeneration after myelosuppressive chemotherapy, but the timing of delivery must be carefully controlled.

Cytokines

Cytokine signaling is also an essential component of the cascade regulating HSC regeneration. A cytokine screen of bone marrow fluid from mice with endothelial cells resistant to irradiation-induced apoptosis identified EGF as a factor promoting radioprotection of HSCs40. EGF receptor signaling in HSCs was able to directly induce multilineage regeneration of a pool of HSCs that survived after myelosuppressive injury by suppressing the proapoptotic protein PUMA, with a skewing toward myeloid recovery over T lymphoid lineages40.

The cytokine pleiotrophin secreted from stromal components has been shown regulate the balance between myeloid and lymphoid cell regeneration after myelosuppression through a β-catenin–independent increase in expression of cyclin D1 (CCND1) and C/EBPα (CEBPA) in Lin−Sca-1+c-Kit+ (LSK) cells94. Associated HSC regeneration after myeloablation due to pleiotrophin may also be mediated through Notch signaling94. Additionally, VEGF is able to induce HSC survival by inhibiting apoptotic death of HSCs caused by irradiation and through an internal autocrine loop mechanism in which only inhibitors that penetrate the intracellular region are able to block receptor signaling, as opposed to surface-binding antibodies124,125. FGF secreted by megakaryocytes promotes HSC proliferation and mobilization through FGF receptor-1 expressed by hematopoietic stem and progenitor cells, which stimulates nuclear factor κB (NF-κB) transcription and upregulation of CXCR4 in response to bone marrow damage126. The inflammatory cytokine IFN-γ has been shown to stimulate quiescent HSCs to proliferate and produce an increase in downstream progenitors while preventing HSC exhaustion in homeostasis and during infectious stress12, although other studies have suggested that IFN-γ impairs HSC maintenance127. Thus, taken together, these studies suggest that distinct sets of cytokines may have more apparent functions during regenerative stress.

Extracellular matrix proteins

A number of extracellular matrix (ECM) and cell adhesion proteins have been implicated as having effects on regeneration. The preference of HSCs to engraft at the endosteal niche as compared to a more central localization is promoted by the calcium-sensing receptor (CaR) expressed on HSCs, leading to enhanced CXCR4 signaling and increased HSC adhesion to collagen I, a predominant component of the bone marrow ECM that is released by mesenchymal cells128. E-selectin, a cell-adhesion molecule expressed constitutively by bone marrow endothelial cells, promotes HSC adhesion to the vascular niche, resulting in their proliferation, may be expressed at higher levels on endothelial cells located near the endosteal region compared with those near the central vein and is found at increasing levels during recovery from irradiation36. Deletion of E-selectin in vivo enhances HSC quiescence and self renewal and HSC survival after chemotherapy or radiation, accelerating blood neutrophil recovery. Although the counterreceptor on HSCs remains unidentified, E-selectin ligand-1 (ESL-1) seems to be a prime candidate, as it mediates mainly E-selectin binding and the homing of LSK cells129.

The ECM protein tenascin C (TNC), which is expressed in stromal and endothelial cells, is notably upregulated during hematopoietic recovery after myeloablation through binding with integrin α9 on the surface of hematopoietic stem and progenitor cells, leading to increased expression of the cell-cycling genes cyclin D1 and cyclin E1 (ref. 130). After transplantation into nonirradiated mouse recipients, increased production of the glycosaminoglycan hyaluronan through Has3 synthase by the blood vessels within the endosteum induces transendothelial migration and HSC homing to the trabecular metaphysis region98. Robo4 expression on HSCs regulates cell location though interaction with the slit family of secreted ligands and cooperates with the CXCR4 receptor in HSCs to mediate HSC anchorage to bone marrow niches. Robo4 is also expressed by endothelial cells and shares a pathway that is regulated by VEGF receptors, which has been shown previously to

promote hematopoietic reconstitution by repairing irradiation-induced damage to the sinusoidal endothelium42,131. Thus, Robo4 expression by endothelial cells might also affect hematopoietic reconstitution ability131.

Niche cell populations in regeneration

Select niche constituents have been shown to directly promote hematopoietic regeneration. Macrophages contribute to recovery from anemia as well as the pathological progression of polycythemia vera and β-thalassemia by modulating erythroid proliferation and differentiation by promoting signaling pathways complementary to Epo-EpoR-Jak2 signaling and by providing iron to regulate erythropoiesis132,133. Megakaryocyte mobilization from the endosteal region to the vascular niche occurs through VEGF-A, which acts with VEGFR1 to induce megakaryocyte maturation and platelet production, leading to CXCR4 upregulation and translocation in the bone marrow134.

Vascular damage after myelosuppressive therapy prevents relocation of megakaryocytes, leading to reduced platelet recovery134,135. Sinusoid-associated nestin-GFPdim MSCs are largely destroyed after chemotherapy, whereas nestin-GFPbright MSCs wrapping arterioles are more quiescent and are chemoresistant. Conditional deletion of NG2-expressing pericytes wrapping the arterioles resulted in cycling of HSCs, indicating that HSC quiescence is maintained near arterioles. The affinity for HSCs and nestin-GFPbright MSCs to colocalize and maintain a quiescent state during homeostasis suggests the potential for arterioles to have an important role in regulating HSC regeneration30. Furthermore, chemotherapy-induced nerve injury has been shown to slow down hematopoietic recovery unless neuroprotection occurs, promoting the survival of nestin+ cells and endothelial cells after chemotherapy through deletion of the tumor suppressor gene p53, production of 4-methylcatechol–induced nerve growth factor (NGF) or injection of glial-derived neurotrophic factor (GDNF), leading to improved hematopoietic recovery1. This vital function of the sympathetic fibers for promoting the survival of niche constituents further points toward arterioles, which are ensheathed with nerve fibers, as a key structural basis for initiating reconstitution and hematopoietic regeneration (Fig. 3).

The aging effect

All HSC progeny are prone to the effects of aging136. Aging negatively influences the maintenance of HSC function by increasing HSC proliferation and promoting a biased differentiation potential (Fig. 4)137,138. Transplantation of HSCs from aged mice into younger recipients revealed that the aged cells had a 50% lower chance of homing to the bone marrow and were less likely to contribute to hematopoiesis compared to the younger cells136. Additionally, differentiation is biased toward the myeloid lineage both in clonal analyses and during transplantation after myeloablation, leading to reduced blood cell reconstitution136. This could also alter immune system function, leading to an increased risk among the elderly for infectious diseases, autoimmune diseases, anemia and ineffective vaccinations136,137,139–142. Although the exact mechanism has not yet been defined to explain this phenomenon, two potentially complementary viewpoints have been suggested136,143.

Intrinsic regulation of aging

One perspective on aging supports an intrinsic regulation of HSCs that results in increased differentiation biased toward the production of common myeloid progenitors and a decreased ability to produce common lymphoid progenitors and erythroid cells142–145. These intrinsic changes lead to stem cell exhaustion and decreased hematopoietic cell repopulation capacity, as well as reduced survival rates146. A downregulation of the cell adhesion molecules α4, α5 and VCAM on aged HSCs leads to reduced adhesion to bone marrow stromal cells expressing high levels of HSC maintenance genes, which might account for the observed increase in mobilization in aged mice138,141,147. TGF-β1 has been reported to enhance myeloid-biased HSC differentiation while inhibiting lymphoid-biased HSCs90. CD41 cell surface expression has been found to increase in aged stem cells that are capable of long-term repopulation and survival and also to shift these cells toward a myeloid lineage bias148. Aryl hydrocarbon receptor (AhR), a helix-loop-helix transcription factor with a role in immunity, has been shown to have a role in HSC regulation in aging, as its depletion led to premature HSC exhaustion, downregulated self-renewal potential in competitive repopulation and serial transplantation and development of a myeloproliferative disorder149.

The deletion from HSCs of Ott1 (one twenty two-1, also called Rbm15), a protein that has been shown previously to regulate hematopoiesis, pre-B cell development and competitive reconstitution150, induced a phenotype similar to that observed in age-associated physiological changes, including loss of quiescence during stress, increased bias toward myeloid cell production, elevated ROS levels, increased mitochondrial mass, DNA damage and increased activation of NF-κB and p38 MAPK151. Additionally, HSCs lacking activated leukocyte cell adhesion molecule (ALCAM) also displayed a phenotype that was consistent with aging, with a diminished capacity for long-term repopulation152.

The chromatin regulator Satb1 is induced during differentiation from HSCs to lymphoid lineage cells, and its expression levels decrease in aged HSCs153, suggesting that its expression levels could potentially be used as an indicator of immunosenescence and aging. There are increased levels of CDC42, a RhoGTPase that regulates cell-cell contact, cell-ECM adhesion and cell polarity144, in aged mice, leading to the onset of an aging phenotype that includes lineage skewing, decreased regenerative capacity, impaired homing, loss of cell polarity and increased mortality144,154. HSC aging was recently attributed to the shift from canonical to noncanonical Wnt signaling as a result of increased Wnt5a expression in aged HSCs, leading to the activation of CDC42 (ref. 155). Interestingly, inhibition of Wnt5a in LT-HSCs using shRNA in aged mice led to a phenotype that was characteristic of young HSCs and could potentially form the basis for rejuvenation therapy155.

ROS can also induce intrinsic cellular changes leading to replicative senescence and aging146. After oxidative stress, increased ROS levels seem to associate with decreased levels of thioredoxin-interacting protein (TXNIP), FOXO depletion, p38-mTOR activation and telomere shortening that leads to DNA damage115,146,156–158. After DNA damage, cell regulation occurs, leading to cell cycle checkpoint activation, apoptosis or

differentiation controlled by p16INK4a, BCL-2, BATF and p53 (refs. 113,159–162). Of these proteins, BCL-2 and p53 have been established as important regulators of apoptosis113. In addition to its role in apoptosis, p53 has a vital role in preventing the expansion of abnormal cells and is involved in numerous DNA damage repair pathways such as activation of cell cycle checkpoints and suppression of homologous recombination113,115.

DNA damage restricted to the mitochondria of mice containing defective mitochondrial polymerase-γ also induces a phenotype similar to premature HSC aging that is characterized by anemia, lymphopenia and myeloid lineage skewing; however, these mice display variations in gene expression profiles compared to true aged mice and show little effect on the size of the HSC pool159. Thus, although mitochondrial DNA mutations might be a contributing factor in physiological aging, they may not be the primary driver of somatic stem cell aging159. The overlapping HSC phenotype between irradiation stressand aging-induced cellular changes (derived from ROS activation and subsequent DNA damage) indicates that irradiation may induce premature aging. This concept, however, needs further validation.

Extrinsic regulation of aging

Another model of aging suggests an extrinsic regulation mediated by the microenvironment, which induces a common myeloid progenitor bias rather than effects caused by age-specific intrinsic HSC changes146. In favor of a microenvironmental balance, HSCs have been shown to change location relative to the bone surface in aged mice compared to young animals and localize further away from the endosteum163. Furthermore, given that bone marrow endothelial cells have been shown to promote HSC regeneration in part through upregulation of adhesion proteins, the reduction in HSC adhesion to bone marrow stromal cells with aging could account for the decrease in self-renewal ability among aged HSCs. Aged mice also had a decreased number of mesenchymal progenitor cells located near the endosteal surface compared to young mice, potentially hampering the ability of HSCs to remain quiescent164. The proinflammatory cytokine CCL5, which is secreted by stromal or differentiated blood cells, may be another important factor promoting extrinsic stem cell regulation, as it induces myeloid lineage skewing in aged HSCs and is associated with a decrease in lymphoid progeny165. Adipocytes are increasingly present in the bone marrow microenvironment with age, which negatively regulate hematopoiesis and delay engraftment53. Systemic increases in the plasma protein concentration of several secreted signaling factors in aged mice could further contribute to the age-associated changes within the bone marrow niche, although the underlying mechanisms remain to be elucidated166.

Further investigations into the interplay between the intrinsic and extrinsic mechanisms involved in regulating HSC aging could lead to a therapeutic basis for promoting stem cell rejuvenation. Intrinsic changes often yield phenotypes that are complementary to extrinsic aging effects, indicating that the HSC aging phenotype involves a complex array of factors and a line of communication between the HSC and its niche.

Future directions

Enormous progress has been made in elucidating the key cellular players responsible for regulating the hematopoietic niche, but many unresolved areas remain. Powerful imaging technologies have led to a more comprehensive understanding of HSC localization, pointing toward vascular niches as the favorable anatomic compartment required for hematopoietic regeneration. Although these technological advances have clarified certain longstanding questions, our understanding of the bone marrow stroma remains nascent, as recently identified genetic markings, including nestin, Lepr and Osx, label a small fraction of bone marrow stromal cells. Thus, the origin, identity and function of the majority of stromal cells are unknown167,168. The identification of markers to define the subsets of stromal cells will improve our understanding of HSC maintenance regulation.

Hematopoietic aging—through both intrinsic and extrinsic mechanisms—invariably impairs regenerative potential; however, rejuvenation remains a treacherous road that may cross paths with malignant transformation. The advent of induced pluripotent stem cells poses a great advance in this regard169, demonstrating that cellular differentiation might be reversible. Pharmacological approaches to targeting age-associated intrinsic pathways or locally targeting extrinsic circulating cytokines could lead to new rejuvenation strategies. Perhaps the HSC subpopulations within the bone marrow do not uniformly age, leading to the protection of certain subsets as reserves. Thus, therapeutics to selectively target and eliminate the population of malfunctioning aged HSCs could lead to a ‘rejuvenation wave’ of transient replication and repopulation of the bone marrow with the remaining primitive HSCs.

Expanding HSCs for regenerative cell therapy is an unmet challenge in need for advancement (Box 1). Currently, numerous culture conditions with variations in growth medium, cytokine or chemical compound supplementation, cocultured cells and varied oxygen tension are used to expand and differentiate HSCs; however, these techniques have also resulted in poor bone marrow engraftment in allogeneic transplantations in mice and humans170–173. Understanding how to generate engraftable HSCs from pluripotent stem cells may give insight into the developmental cues uncoupling HSC quiescence and self renewal. Improved in vitro models of bone marrow niches through combinations of biomimetic biomaterial substrates, bioreactors, coculture of multiple candidate niche cells and real-time imaging could give rise to a new understanding of niche cell functionality ex vivo173. This knowledge, combined with an improved understanding of the molecular mechanisms controlling HSC self renewal, could potentially lead to new expansion protocols.

Improving the regeneration capacity of HSCs in patients with recurring cancers subjected to irradiation or chemotherapy is another area in need of improvement. Chemotherapeutic treatment for patients with cancer causes acute bone marrow injury followed by aplasia and bone marrow remodeling, leading to impaired hematopoietic reserve and function (Box 3). Sympathetic nerves seem to promote the survival of key niche components leading to hematopoietic recovery1, indicating that additional investigation into the specific stromal cells acted on by the sympathetic nerves may improve therapies aimed at increasing bone

marrow functionality. The development of approaches to radioprotect perivascular stromal cells and endothelial cells could also further improve hematopoietic reconstitution. Therapeutic delivery of cytokines including FGF, EGF and IFN-γ, repression of proapoptotic proteins such as PUMA, delivery of the antiapoptotic factor aPC110 or inhibition of regeneration-hindering pathways such as TGF-β might also aid in augmenting multilineage hematopoietic reconstitution. However, it remains unclear whether a 'magic bullet' promoting HSC expansion without impinging on self renewal exists.

Similarities among varying tissues containing stem cell niches, such as the intestinal crypt, hair and skin174–176, may allow us to extrapolate benefits to hematopoietic maintenance and regeneration. Continued progress will undoubtedly lead to an enhanced understanding of the key players in niche function while technological advancements in imaging and construction of artificial ex vivo niches will provide exciting new possibilities for improved regenerative therapies and rejuvenation strategies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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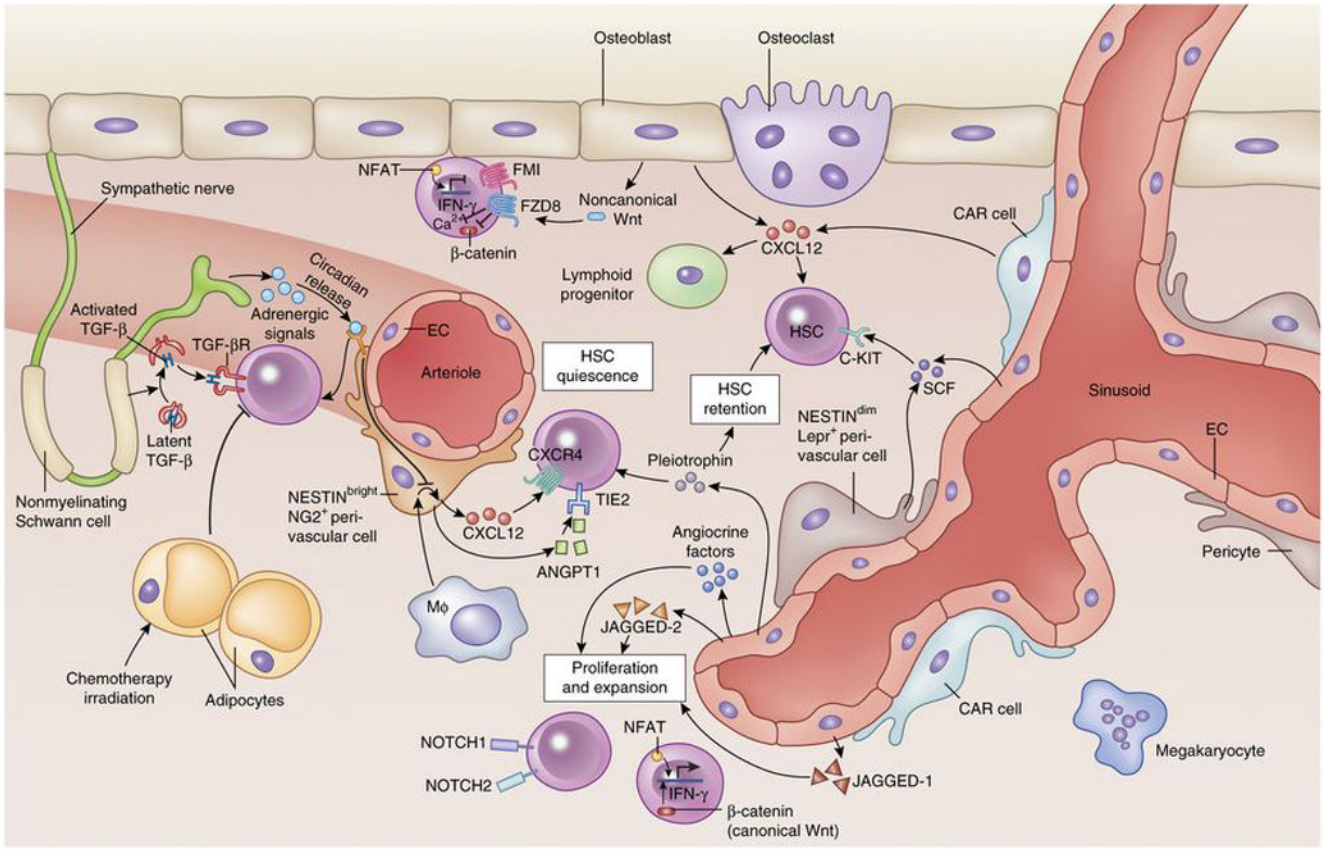

Figure 1.

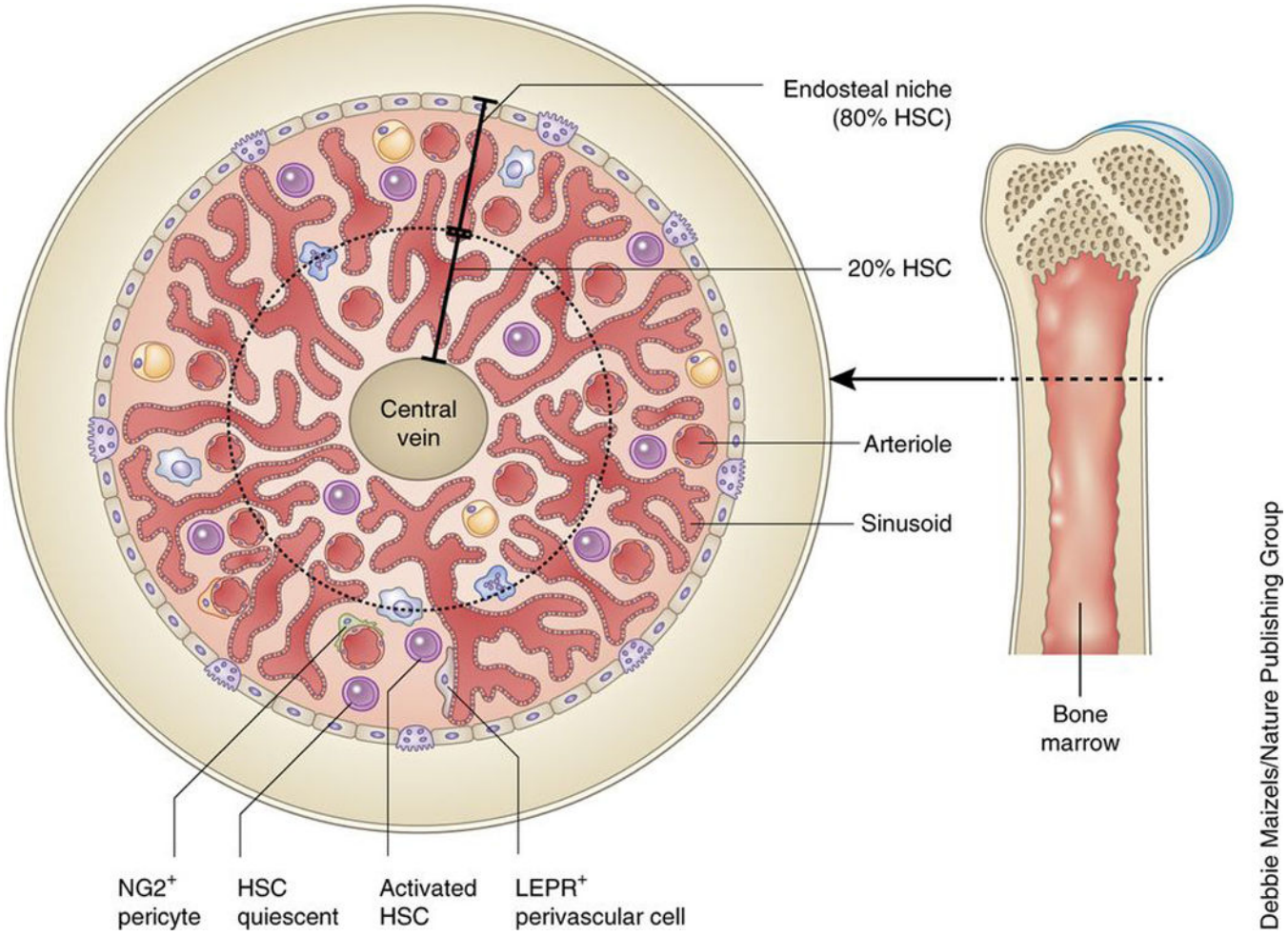

Figure 2.

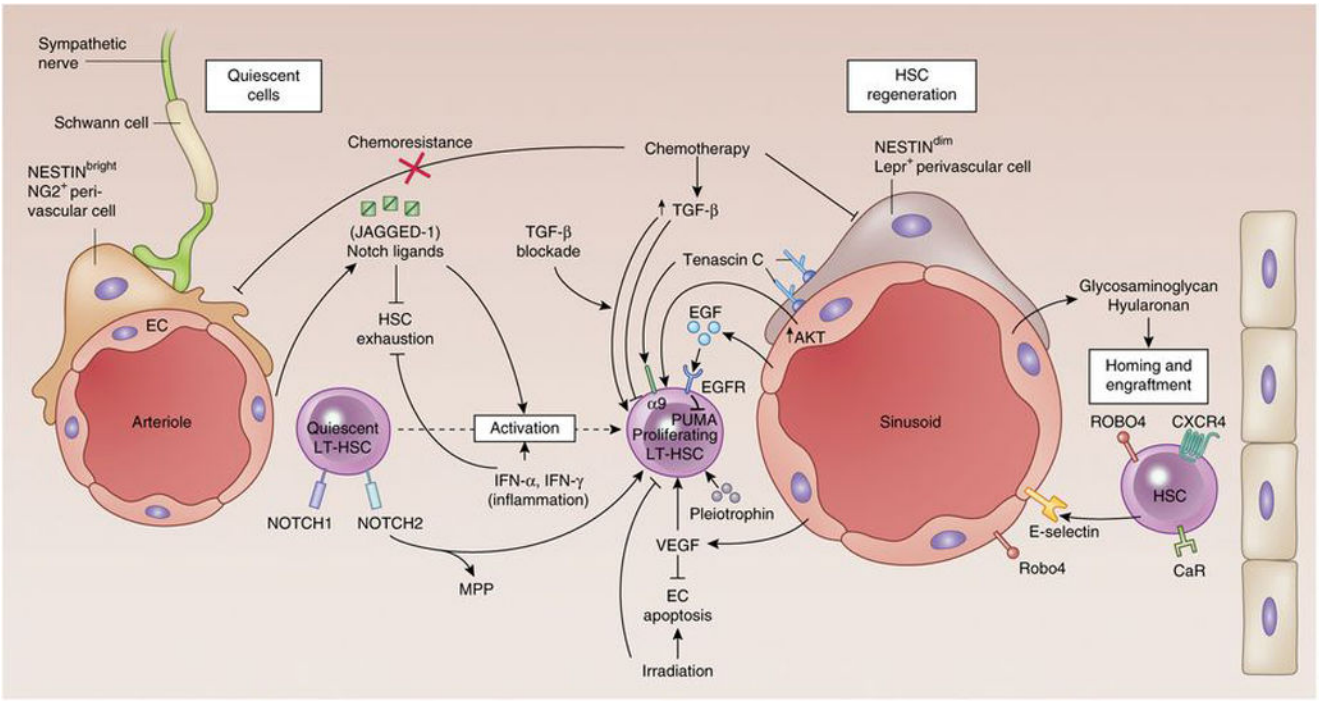

Figure 3.

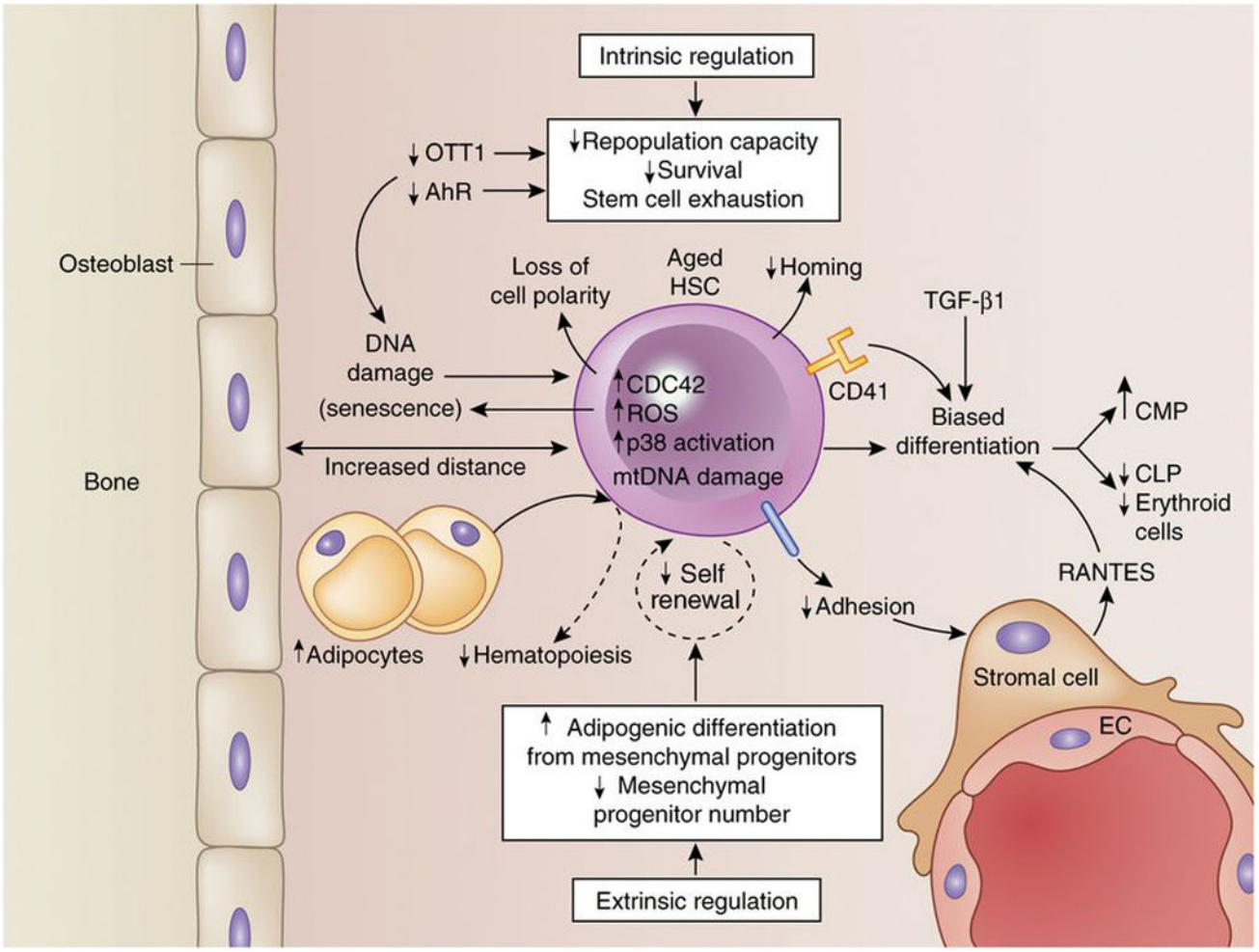

Figure 4.