Adult haematopoietic stem cell niches

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Abstract | Stem cell niches are specialized microenvironments that promote the maintenance of stem cells and regulate their function. Recent advances have improved our understanding of the niches that maintain adult haematopoietic stem cells (HSCs). These advances include new markers for HSCs and niche cells, systematic analyses of the expression patterns of niche factors, genetic tools for functionally identifying niche cells *in vivo*, and improved imaging techniques. Together, they have shown that HSC niches are perivascular in the bone marrow and spleen. Endothelial cells and mesenchymal stromal cells secrete factors that promote HSC maintenance in these niches, but other cell types also directly or indirectly regulate HSC niches.

Haematopoiesis
The process by which blood cells and immune system cells — including erythrocytes, platelets and white blood cells — are formed from haematopoietic stem cells, which undergo lineage restriction and then differentiation by giving rise to various restricted haematopoietic progenitors.

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Stem cell niches are local tissue microenvironments that maintain stem cells and regulate their function by producing factors that act directly on stem cells[1]. Long-range factors produced by other tissues can also affect stem cells and their niche, but these are not the main focus of this Review. The identification of stem cell niches and the mechanisms by which they regulate stem cells offers the opportunity to better understand tissue homeostasis and regeneration.

Extensive work has been carried out to identify and characterize niches for haematopoietic stem cells (HSCs), which are rare, self-renewing and multipotent progenitors that sustain haematopoiesis. Haematopoiesis is required for the ongoing production of blood cells and immune cells — including erythrocytes, platelets and white blood cells — throughout life. To generate these cells, HSCs give rise to an array of restricted progenitors, which proliferate extensively and then differentiate into mature cells. Without haematopoiesis, we would not be able to maintain blood cell counts and would die within weeks as a result of anaemia (due to erythrocyte depletion), bleeding (due to platelet depletion) and infection (due to the depletion of myeloid and lymphoid immune effector cells). Although restricted progenitors are responsible for most steady-state haematopoiesis, HSCs must be maintained throughout life to replenish these progenitors[2–5], and to regenerate haematopoietic cells after stresses such as severe infection or blood loss[6].

HSCs arise during embryonic development and occupy a series of niches in fetal tissues where they expand in number and initiate haematopoiesis (see BOX 1 for a brief summary of fetal haematopoiesis) before localizing primarily to the bone marrow postnatally. No single article could comprehensively cover all of the work on HSC niches. This Review focuses on the location, cellular composition and regulation of adult HSC niches, particularly in the bone marrow. We hope that this will prove to be useful for researchers who have an interest in haematopoiesis and HSC biology, clinicians who have an interest in bone marrow transplantation, and stem cell biologists studying niches in other tissues.

Although the main site of haematopoiesis in adult mammals under normal circumstances is the bone marrow, haematopoiesis can transiently expand into facultative niches in extramedullary tissues, such as the liver and the spleen, in response to severe haematopoietic stresses. Our understanding of the bone marrow and extramedullary HSC niches has advanced rapidly in the past 5 years as a result of new markers for HSCs and niche cells, new imaging techniques for niches, functional genetic analyses of the sources of crucial niche factors, and the identification of new mechanisms by which the niche regulates HSC function. These studies have been carried out mainly in mice and zebrafish. HSC niches in humans are presumed to be similar, as most aspects of HSC biology and haematopoiesis are similar in mice and humans. Nonetheless, there are few data so far from humans.

HSCs are the most clinically used type of stem cell as they are the main active ingredient in bone marrow, cord blood and mobilized peripheral blood transplants. These forms of bone marrow and HSC transplantation are potentially curative for patients with certain disorders that involve bone marrow failure (such as Fanconi anaemia), haematopoietic malignancies (such as acute myeloid leukaemia) and benign haematopoietic disorders (such as severe combined immunodeficiency and sickle cell anaemia). Before transplantation, patients are typically given a conditioning regimen to ablate endogenous haematopoietic cells (a process also known as myeloablation) and to enable the engraftment of transplanted HSCs. This leads to a period of pancytopenia before the transplanted cells adequately restore haematopoiesis. Consequently, transplant recipients

Box 1 | **HSCs are closely associated with blood vessels during development**

Genetic tools and improved imaging techniques for studying mouse and zebrafish embryos have increased our understanding of haematopoietic stem cell (HSC) niches during development.

Fetal haematopoiesis in mammals

HSCs arise from the haemogenic endothelium[145–148], which is associated with blood vessels in embryonic and extra-embryonic sites within the mammalian embryo; these sites potentially include the dorsal aorta[145–150], the umbilical and vitelline arteries[147,149], the yolk sac[147] and the placenta[151,152]. Haematopoiesis first occurs in the yolk sac, and then in the liver, spleen and bone marrow by the time of birth[153]. The major expansions in HSC numbers during development occur in the placenta[151] and then in the fetal liver[154]. Each of these tissues contains HSC niches that are likely to be associated with blood vessels.

The fetal liver HSC niche

40% of fetal liver HSCs are located within 20 μm of portal blood vessels, which are associated with pericytes that express nestin and neural–glial antigen 2 (NG2)[155]. Diphtheria toxin-mediated depletion of *Ng2*-Cre$^+$ stromal cells reduces the frequency of dividing HSCs in the fetal liver but increases the reconstituting capacity of fetal liver cells upon transplantation into irradiated mice[155]. This suggests that NG2$^+$ cells are required for HSC proliferation or for the maintenance of a subset of dividing HSCs in the fetal liver. The conditional deletion of *Scf* (which encodes stem cell factor) using *Ng2*-Cre also significantly reduced HSC frequency in the liver during fetal development, although no data have been published from transplantation assays in relation to whether this increased or decreased the reconstituting potential of fetal liver cells[79].

Imaging advances in zebrafish

Recent studies of zebrafish embryos have visualized the interactions of HSCs with vascular and perivascular cells *in vivo*[156–158]. Confocal time-lapse imaging confirmed that HSCs arise from haemogenic endothelial cells in the zebrafish dorsal aorta[145]. Macrophages in the mesenchyme surrounding the dorsal aorta facilitate the intravasation of HSCs into the circulation by secreting matrix metalloproteinases[159]. HSCs then migrate through the blood to the caudal haematopoietic tissue, which is a site of haematopoiesis in zebrafish larvae, before transitioning to the kidney in adult fish[160]. In the caudal haematopoietic tissue, HSCs migrate through the vessel wall and lodge adjacent to CXC-chemokine ligand 12 (CXCL12)-expressing mesenchymal stromal cells, and this is followed by endothelial cell remodelling to form a pocket that surrounds the HSCs[156]. This niche seems to be analogous to mammalian HSC niches[17,78,84], as both mouse and zebrafish HSCs are maintained in their niches by endothelial cells and CXCL12$^+$ mesenchymal stromal cells. The stromal cells seem to orient HSC divisions[156], whereas the endothelial cells secrete cytokines that promote HSC maintenance, including SCF[161]. Future studies will be required to characterize the HSC niche in the adult zebrafish kidney and to assess whether it is also similar to mammalian HSC niches.

are at risk of serious complications, including anaemia, bleeding and opportunistic infections[7]. The safety of HSC transplantation has been increased in recent years by the provision of improved supportive care to minimize these complications, and by strategies to increase the speed and likelihood of successful engraftment. Ongoing advances in our understanding of the HSC niche offer the opportunity to reduce the toxicity of conditioning regimens, to further accelerate HSC engraftment, to promote haematopoietic regeneration from endogenous progenitors and to promote the *ex vivo* expansion of HSCs.

HSC transplantation
A potentially curative therapy involving the replacement of a patient's blood-forming cells with those from a donor (allogeneic) or with their own cells (autologous) that had been stored before chemotherapy or radiation treatment. This procedure can be carried out by transplanting whole bone marrow cells or enriched populations of haematopoietic stem cells (HSCs), which can be obtained from various sources, including bone marrow, mobilized peripheral blood or umbilical cord blood.

HSC localization in adult bone marrow

The idea that HSCs occupy a specialized niche in haematopoietic tissues was first proposed in 1978 (REF. 8); however, it took approximately 25 years to develop the tools required to image HSC localization with confidence in haematopoietic tissues (as reviewed in REF. 9). The fundamental problem was that identifying HSCs by flow cytometry required a combination of approximately a dozen antibodies conjugated to approximately five different fluorochromes. This labelling protocol was too complex for the identification of HSCs in tissue sections by immunofluorescence analysis. The discovery of signalling lymphocyte activation molecule family markers (SLAM family markers) for HSCs made it possible to highly purify HSCs (as CD150$^+$CD48$^-$CD41$^-$ cells) using a simple, two-colour combination of antibodies[10]. Analysis of the localization of these cells in tissue sections showed that most HSCs reside adjacent to sinusoidal blood vessels in the bone marrow and spleen (FIG. 1). Based on this observation, we proposed the existence of a perivascular niche for HSCs[10].

Studies conducted by other laboratories using alternative combinations of HSC markers confirmed that most HSCs localize adjacent to sinusoidal blood vessels[11–13]. New genetic markers, including *Ctnnal1* (which encodes α-catulin)[14] and *Hoxb5* (which encodes homeobox b5)[15], have further simplified the identification of HSCs and refined our understanding of their localization. In *Ctnnal1*-GFP knock-in mice, green fluorescent protein (GFP) is expressed by only 0.02% of bone marrow haematopoietic cells, including all or almost all HSCs[14], and *Ctnnal1*-GFP$^+$KIT$^+$ cells have a level of HSC purity that is comparable to cells identified by the best combinations of HSC markers[14]. KIT is the receptor for stem cell factor (SCF; also known as KIT ligand), a growth factor that is required for HSC maintenance[16,17]. The use of *Ctnnal1*-GFP and KIT in combination with tissue clearing and deep confocal imaging made it possible to digitally reconstruct large 3D segments of bone marrow and assess the localization of thousands of HSCs[14]. Approximately 80% of dividing and non-dividing HSCs are most closely associated with sinusoidal blood vessels, with another 10% of HSCs being adjacent to arterioles, and 10% being adjacent to transition zone vessels (see FIG. 1 for a description of the bone marrow vasculature). Only a small percentage of HSCs are located at the endosteum.

HSCs can also be highly purified on the basis of *Hoxb5* expression[15]. An analysis of HSC localization using *Hoxb5*-mCherry knock-in mice confirmed that HSCs are consistently perivascular[15]. Multiple distinct sets of markers that each identify HSCs to a high level of purity thus show that HSCs reside perivascularly, mainly around sinusoidal blood vessels, in adult bone marrow. Each of these marker sets identifies cell populations that are overwhelmingly quiescent.

Multiple other genetic markers have been identified that are highly restricted to HSCs, although they have not yet been used to study the HSC niche. Bone marrow cells that express high levels of a *Tie2*-GFP transgene (which labels with GFP those cells that express protein tyrosine kinase with immunoglobulin and EGF homology domains 2) are very highly enriched for HSC activity, and some of these cells localize slightly closer to arterioles than do HSCs that are negative for

Conditioning regimen
Chemotherapy and/or radiation that ablates endogenous haematopoietic cells before haematopoietic stem cell (HSC) transplantation to facilitate the engraftment of the transplanted HSCs.

Ex vivo expansion
Increasing the number of haematopoietic stem cells (HSCs) that are available for transplantation by growing them in culture. For reasons that are not currently understood, it is not yet possible to considerably or sustainably increase the numbers of HSCs in culture, despite decades of effort.

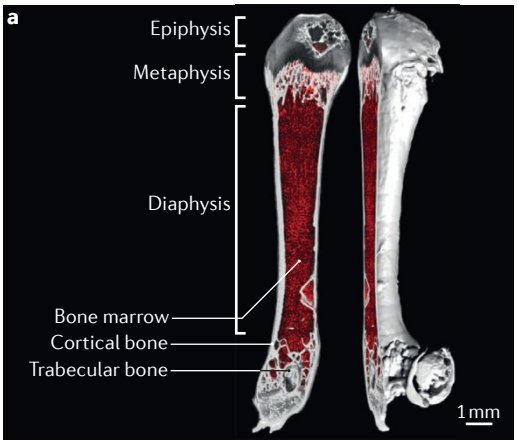
Signalling lymphocyte activation molecule family markers
(SLAM family markers). A group of cell-surface receptors (including CD150, CD48, CD229 and CD244) that are differentially expressed among haematopoietic stem cells (HSCs) and other haematopoietic progenitors in a manner that enables them to be used to identify HSCs and multipotent progenitors.

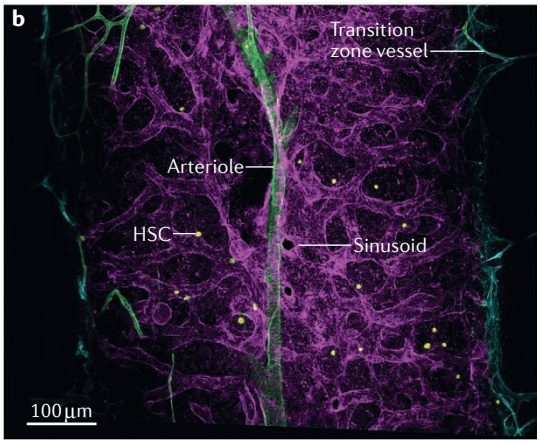
Sinusoidal blood vessels
Fenestrated venous blood vessels that are found in haematopoietic tissues and through which haematopoietic cells can migrate into and out of the circulation.

Perivascular niche
A microenvironment in the bone marrow that is located adjacent to a blood vessel and that supports the maintenance of haematopoietic stem cells and/or other haematopoietic progenitors.

Ctnnal1-GFP knock-in mice
A gene-targeted mouse line in which green fluorescent protein (GFP) is knocked into the *Ctnnal1* locus (which encodes α-catulin) such that GFP marks α-catulin$^+$ cells, which are highly enriched for haematopoietic stem cells.

Arterioles
In the context of this Review, blood vessels of variable diameter that carry arterial blood into the bone marrow.





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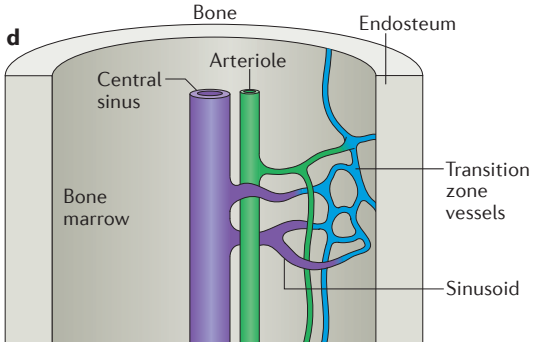
	Vessel diameter	Basal lamina	Position
Arterioles	Variable	Continuous	Parallel to the long axis
Transition zone vessels	Small	Continuous	Outer 20% of marrow volume closest to the bone surface
Sinusoids	Large	Fenestrated	Perpendicular to the long axis



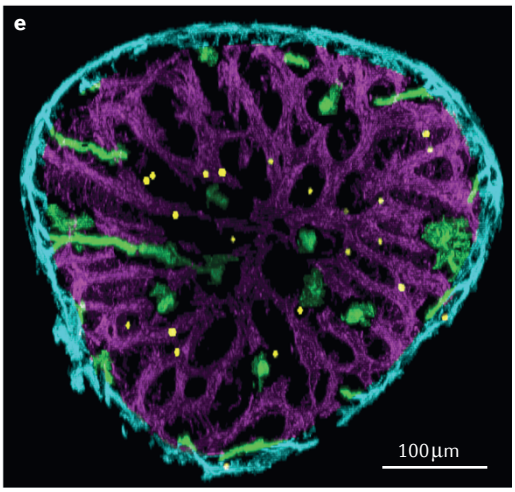


Figure 1 | **The vasculature in the bone marrow and its relationship to HSCs. a** | A bisected mouse femur showing cortical and trabecular bone, as well as the bone marrow cavity. **b** | A higher magnification image of the vasculature in the bone marrow showing the distribution of α-catulin$^+$ haematopoietic stem cells (HSCs; yellow; these cells were also selected for being KIT$^+$) relative to sinusoids (purple), arterioles (green) and transition zone vessels (blue). **c** | Blood vessels in the bone marrow can be distinguished on the basis of diameter, morphology, and whether the basal lamina surrounding the blood vessel is continuous or fenestrated. **d** | In long bones, such as the tibia and femur, arterioles (green) branch from the nutrient arteries that carry blood into the bone marrow and run longitudinally through progressively smaller arterioles near the endosteal surface. These smaller arterioles become transition zone vessels (blue), which connect arterioles to sinusoids and have properties that are intermediate between arterioles and sinusoids. Sinusoids (purple) are larger-diameter vessels that return venous blood into the central sinus, which carries it out of the bone marrow. **e** | A cross-section of the tibial diaphysis showing that HSCs (yellow) are widely distributed throughout the bone marrow, but are modestly enriched in the central marrow and depleted towards endosteal surfaces[14]. The images in parts **a**, **b** and **e** were generated by the laboratory of S.M. for this Review. Parts **c** and **d** are reproduced with permission from REF. 14, Macmillan Publishers Limited.

Transition zone vessels
In the context of this Review, small blood vessels that connect arterioles to sinusoids in the bone marrow and that are located near the endosteum.

Endosteum
The internal bone surface at the interface between bone and bone marrow.

Hoxb5-mCherry knock-in mice
A gene-targeted mouse line in which the fluorescence marker mCherry is knocked into the *Hoxb5* locus (which encodes homeobox b5) such that mCherry marks *Hoxb5*$^+$ cells, which are highly enriched for haematopoietic stem cells.

CXCL12-abundant reticular cells
(CAR cells). Stromal cells in the bone marrow that are mainly associated with sinusoidal blood vessels and that express high levels of CXC-chemokine ligand 12 (CXCL12), which is an important factor for retaining haematopoietic stem cells in the bone marrow niche.

Leptin receptor-expressing cells
(LEPR$^+$ cells). Stromal cells in the bone marrow that are mainly associated with sinusoidal blood vessels and that express high levels of the crucial haematopoietic stem cell niche factors stem cell factor and CXC-chemokine ligand 12. These cells have commonly been identified using *Lepr*-Cre recombination systems, but in young-adult bone marrow nearly all of the bone marrow cells that recombine with *Lepr*-Cre also stain positively with LEPR-specific antibody.

Nes-CreER$^+$ cells
Cells that express a tamoxifen-activated form of Cre recombinase under the control of regulatory elements of *Nes* (which encodes nestin). The conditional reporter is expressed by rare periarteriolar stromal cells in adult bone marrow but is more widely expressed in early postnatal bone marrow, where it shows widespread expression in endothelial cells, for example.

Tie2-GFP, although no data have been published on their position relative to sinusoids[18]. Cells expressing an *Fgd5*$^{mCherry/+}$ knock-in reporter[19] (which labels with mCherry those cells that express FYVE, RhoGEF and PH domain-containing 5) and cytokeratin 7-expressing cells[20] are also highly enriched for HSCs, although no data have been published on the localization of these cells in the bone marrow.

Cell ablation to identify niche cells

The earliest studies that functionally tested whether particular cell types regulate HSC maintenance used mice that were engineered to conditionally express herpesvirus thymidine kinase or diphtheria toxin receptor in specific cell populations, and were then administered ganciclovir or diphtheria toxin, respectively, to ablate these cell populations. Such studies ablated several stromal cell populations in the bone marrow, including osteoblasts[21,22], CXCL12-abundant reticular cells (CAR cells)[23], leptin receptor-expressing cells (LEPR$^+$ cells)[24], *Nes*-CreER$^+$ cells[25] (*Nes* encodes nestin) and *Ng2*-CreER$^+$ cells[13] (*Ng2* (also known as *Cspg4*) encodes neural–glial antigen 2), as well as a few types of haematopoietic cell, such as megakaryocytes[26,27] and macrophages[28–30] (TABLE 1). In each case, HSCs entered the cell cycle and/or became depleted after ablation of the indicated cell populations. However, to our knowledge, no cell type has ever been ablated from the bone marrow without inducing HSC activation or depletion. It is therefore not clear whether these observations reflect direct effects of the ablated cells on HSCs. Perhaps the ablation of any cell type in the bone marrow induces an injury response that activates and/or depletes HSCs in a niche-independent manner.

The consequences of osteoblast ablation are particularly informative. In several studies, a rat collagen promoter was used to drive thymidine kinase expression in osteoblasts such that the treatment of these mice with ganciclovir depleted osteoblasts[21,22,31]. Osteoblast ablation acutely depleted early lymphoid progenitors from the bone marrow, which suggests that these cells depend on osteoblasts for their maintenance[21,22]. HSCs also became depleted, but only weeks after osteoblast ablation when the bone marrow became pancytopenic. Given that there was no acute effect of osteoblast ablation on HSC frequency[21,22], that few HSCs reside close to bone surfaces[14] and that osteoblasts do not express the crucial niche factor SCF[17], osteoblasts do not seem to directly promote HSC maintenance[9]. Rather, there may be widespread crosstalk in the bone marrow by which many cells indirectly regulate other cells. This may explain why osteoblast ablation eventually leads to pancytopenia even though osteoblasts are not required in other haematopoietic tissues (such as the liver and spleen) for HSC maintenance or haematopoiesis. The potential for widespread indirect effects of cell ablation is even more pronounced when vascular cells are ablated, owing to the disruption of blood flow through the bone marrow (for example, *Nes*-CreER is expressed by bone marrow endothelial cells[25,32]).

Growth factors required for HSC maintenance

An alternative approach to identifying niche cells is to identify the cells that synthesize the factors that promote HSC maintenance. A few growth factors are known to be non-cell-autonomously required for HSC maintenance, including SCF[16,33,34], CXC-chemokine ligand 12 (CXCL12)[12,35–37] and thrombopoietin[38–41].

SCF. SCF binds to the receptor KIT[42–49], which is expressed by HSCs[50]. Both *Scf* and *Kit* are required for HSC maintenance[16,34,51]. SCF is present in both membrane-bound and soluble forms, each of which activates signalling by the receptor KIT[42–49]. The membrane-bound form of SCF seems to be important for HSC maintenance as HSCs are depleted in *Sl/Sl*^{d} mutant mice[16], which express soluble SCF but lack the membrane-bound form[52]. Mice with a mixture of wild-type and *Sl/Sl*^{d} stromal cells exhibit normal haematopoiesis only in the immediate vicinity of the wild-type cells, which indicates that SCF functions locally to create the niche[53]. Niche cells would thus be expected to express SCF, and HSCs would be expected to have direct contact with these cells. Other early haematopoietic progenitors, as well as mast cells, also depend on SCF–KIT signalling, although it remains unclear whether they derive this signal from the same niche cells as do HSCs.

CXCL12. CXCL12 promotes HSC maintenance and retention in the bone marrow by activating signalling through CXC-chemokine receptor 4 (CXCR4), which is expressed by HSCs[54]. CXCL12–CXCR4 signalling also regulates HSC engraftment after transplantation and HSC proliferation[55,56]. Global conditional deletion of *Cxcl12* or *Cxcr4* depletes HSCs from adult bone marrow[12,57]. HSC niche cells must, therefore, express CXCL12 in addition to SCF. CXCL12–CXCR4 signalling also regulates the proliferation and retention of multiple restricted progenitor populations in the bone marrow, including various myeloid and lymphoid progenitors[35,55,58,59].

Thrombopoietin. Thrombopoietin activates signalling by myeloproliferative leukaemia protein (MPL; also known as TPOR) on HSCs. Both thrombopoietin and MPL are required for HSC maintenance[38–41], as well as for megakaryocyte and platelet production[60–62]. Thrombopoietin is expressed at high levels in the liver and to a lesser extent in kidney, and there is limited expression in the bone marrow under normal circumstances[60,63]. It has not been tested whether HSC maintenance is promoted by thrombopoietin that is produced locally in the bone marrow or at a distant site, such as in the liver. The identification of the cellular sources of the thrombopoietin that is required for HSC maintenance is an important area for future research.

Other factors. Many other factors in the bone marrow non-cell-autonomously modulate HSC function but are not necessarily required for HSC maintenance (TABLE 2). Several growth factors that are not required for HSC maintenance or haematopoiesis under steady-state conditions nonetheless promote haematopoietic

Table 1 | **Bone marrow cell types associated with the HSC niche**

Cell population	Percentage of total bone marrow cells	Location	Included cell types	Time when present
Adipocytes[162]	Rare in most bones; can increase with age and irradiation	Can be present throughout bone marrow, and are abundant in the tail vertebrae of mice	Perilipin$^+$ and Oil Red O$^+$ cells, and cells marked by the expression of *Adipoq*-CreER	Become increasingly abundant during adulthood
CAR cells[12]	0.3%[23]	Perivascular; mainly perisinusoidal but also periarteriolar	Nearly all cells that express high levels of CXCL12 and SCF; skeletal stem cells; and LEPR$^+$ cells	Characterized in adult bone marrow
Endothelial cells[91,96,97]	0.01–0.03%[17], depending on dissociation conditions	Blood vessels throughout the bone marrow, including sinusoids and arterioles	CD31$^+$VE-cadherin$^+$VEGFR1$^+$VEGFR2$^+$ cells, and laminin$^+$ cells marked by the expression of *Tie2*-Cre	Beginning in the late fetal stage and persisting throughout adulthood
HSCs[10]	0.007%[10]	Mainly associated with sinusoids, with a smaller proportion present near arterioles[10,13,14]	CD150$^+$, CD48$^-$, KIT$^+$ (REF. 10) and SCA1$^+$ cells, *Ctnnal1*-GFP$^+$ cells[14] and *Hoxb5*-mCherry$^+$ cells[15]	Colonize the bone marrow during late fetal development then persist throughout adulthood
LEPR$^+$ stromal cells[17]	0.3%[24,163]	Perivascular; mainly perisinusoidal but also periarteriolar	Nearly all cells that express high levels of CXCL12 and SCF, including skeletal stem cells	Beginning in postnatal bone marrow and persisting in adult bone marrow
Megakaryocytes[26,27]	~0.1%[164]	Closely associated with sinusoids throughout the bone marrow	CD41$^+$ cells, and cells marked by the expression of *Cxcl4*-Cre	Present at all postnatal and adult stages
Monocytes and macrophages[29,30,165]	~0.4%[166]	Throughout the bone marrow	CD11b$^+$F4/80$^+$ cells, including DARC$^+$ (REF. 30) and SMA$^+$ (REF. 111) subsets, osteoclasts and CD169$^+$ macrophages[165]	Present at all postnatal and adult stages
Nes-CreER$^+$ stromal cells[25]	0.001%[24]	Periarteriolar in adult bone marrow	Endothelial cells and periarteriolar stromal cells[24,25,32]	Common in early postnatal bone marrow, but rare in adult bone marrow[32]
Nes-GFP$^+$ stromal cells[13,25,89]	0.08%[25]	Includes *Nes*-GFP^{low} perisinusoidal cells and *Nes*-GFP^{hi} periarteriolar cells	*Nes*-GFP^{low} cells include LEPR$^+$/CAR cells; *Nes*-GFP^{hi} cells include stromal cells, Schwann cells and endothelial cells[32]	Characterized in fetal[89] and adult[25] bone marrow
NG2$^+$ stromal cells[13]	0.003%[24]	Periarteriolar, as well as associated with nerve fibres and the endosteum in adults; broader distribution in neonates[79]	Stromal cells, osteoblasts, Schwann cells, osteocytes and chondrocytes	Fetal liver[155] and adult bone marrow[13]
Neurons, specifically nerve fibres[77,106,107,167]	Rare	Usually associated with arterioles in central bone marrow[13,90]; rare synapses on some perivascular stromal cells[110]	PGP9.5$^+$ and neurofilament$^+$ cells, including neurons (specifically their processes), Schwann cells, perineurial cells and fibroblasts	Persist throughout postnatal life in the bone marrow
Osteoblasts	Predominant cell type along the endosteum	Bone-lining endosteal cells	Alkaline phosphatase$^+$, osteocalcin$^+$ and CD10$^+$ cells, and cells marked by the expression of *Col1a1*-Cre, *Col1a1*-CreER, *Col1a1*-GFP, *Runx2*-CreER, *Osx*-CreER or *Ocn*-Cre	Arise perinatally and persist throughout adulthood
PRRX1$^+$ stromal cells[84]	0.3%	In limb bones but not in the axial skeleton[163,168]; perivascular and endosteal	LEPR$^+$/CAR cells, osteoblasts, and skeletal stem cells in limb bones	Arise during fetal development and persist in adult bone marrow
Regulatory T cells[169]	~1% of human bone marrow[170]	Widely dispersed but commonly near endosteal surfaces	CD4$^+$CD25$^+$FOXP3$^+$ cells and *Foxp3*-GFP$^+$ cells[169]	Characterized in adult bone marrow
Schwann cells[109,167]	Rare	Associated with nerve fibres	Myelinating and non-myelinating Schwann cells; GFAP$^+$ and S100$^+$ cells; and cells marked by the expression of *P0*-Cre, *Nes*-Cre and *Wnt1*-Cre	All postnatal stages
Skeletal stem cells[98]	0.04%[101,103]	Many in the metaphysis[103,104]	CD51$^+$, PDGFR$^+$ and CD200$^+$ cells[103]; CD146^{hi} cells in humans[100]; and CXCL12^{hi}, SCF^{hi} and LEPR$^+$ cells	Adult bone marrow; different waves of progenitors in fetal and early postnatal bone marrow

Adipoq, adiponectin; CAR cell, CXCL12-abundant reticular cell; *Col1a1*, collagen type I α1 chain; CreER, tamoxifen-activated form of Cre recombinase; *Ctnnal1*, α-catulin; *Cxcl4*, CXC-chemokine ligand 4; CXCL12, CXC-chemokine ligand 12; DARC, Duffy antigen receptor for chemokines; FOXP3, forkhead box protein P3; GFAP, glial fibrillary acidic protein; GFP, green fluorescent protein; HSC, haematopoietic stem cell; *Hoxb5*, homeobox b5; LEPR, leptin receptor; *Nes*, nestin; NG2, neural–glial antigen 2; *Ocn*, osteocalcin; *Osx*, osterix; PDGFR, platelet-derived growth factor receptor; *P0*, protein 0; PGP9.5, protein gene product 9.5 (also known as UCHL1); PRRX1, paired-related homeobox 1; *Runx2*, runt-related transcription factror 2; SCA1, spinocerebellar ataxia type 1 protein (also known as ataxin 1); SCF, stem cell factor; SMA, smooth muscle actin; *Tie2*, tyrosine kinase with Ig and EGF homology domains 2 (also known as *Tek*); VE-cadherin, vascular endothelial cadherin; VEGFR, vascular endothelial growth factor receptor.

Table 2 | **Locally acting factors in adult bone marrow that modulate HSC or niche function but may not be required for HSC maintenance**

Factor	Source	Receptor	Responding cell type or types	Effect on niche or haematopoiesis	Physiological role
Angiogenin[64]	Mesenchymal stromal cells and haematopoietic cells	Secreted RNase that changes RNA processing in a cell-type-specific manner	HSCs, LSK cells and myeloid progenitors	Promotes quiescence in HSCs and the proliferation of myeloid progenitors	Promotes the recovery of haematopoiesis after myeloablation
Angiopoietin 1 (REF. 101)	HSCs, KIT$^+$ cells and LEPR$^+$ cells	TIE2	Endothelial cells	Promotes the regeneration of patent blood vessels after vascular injury	Regulates regeneration of the niche; not required for HSC maintenance or haematopoiesis
Angiopoietin-like protein 3 (REF. 65)	Sinusoidal endothelial cells and liver cells	Unknown	HSCs	Promotes the maintenance of HSCs and some types of restricted haematopoietic progenitor	Dispensable for steady-state haematopoiesis but promotes recovery after myeloablation; also regulates blood lipids; not clear if the effect on HSCs is direct
BMPs	Osteoblasts, endothelial cells and megakaryocytes	Complexes of BMP type I and type II receptors	HSCs	Canonical signalling does not affect HSC maintenance or haematopoiesis[171,172]; BMP4 is required for normal niche function[173]	Dispensable for normal haematopoiesis and HSC frequency, but regulate HSC engraftment after transplantation
FGF1 (REFS 27,66)	Megakaryocytes[27]	FGFR1 (REF. 66) and potentially others	HSCs and megakaryocytes	Promotes the regeneration of HSCs and megakaryocytes after myeloablation	Dispensable for normal haematopoiesis but promotes recovery after myeloablation
FGF2 (REF. 67)	Bone marrow stromal cells	Multiple FGFRs are present on HSCs	HSCs	Promotes the proliferation of HSCs and stromal cells after myeloablation	Dispensable for normal haematopoiesis but promotes recovery after myeloablation
G-CSF	Monocytes and macrophages[174], and various bone marrow stromal cell populations[129]	G-CSF receptor	Stromal cells[130] and myeloid progenitors	Mobilizes HSCs and increases myelopoiesis[124,130]	Mobilizes HSCs in response to sepsis and other inflammatory conditions[124]
IL-6 (REF. 68)	T cells and macrophages	IL-6 receptor	HSCs and restricted progenitors	Promotes HSC self-renewal and regulates the function of restricted progenitors	Dispensable for normal haematopoiesis but required for regeneration after myeloablation
Notch ligands	Bone marrow stromal cells, endothelial cells[175] and osteoblasts	Notch receptors, particularly Notch 2 (REF. 69)	HSCs, progenitors and endothelial cells	Notch 2 promotes HSC regeneration after myeloablation[69]	Dispensable for normal haematopoiesis[69]
Osteopontin[176]	Osteoblasts	Integrins and CD44	HSCs	Negatively regulates HSC frequency	Dispensable for normal haematopoiesis
Pleiotrophin[70]	Endothelial cells and LEPR$^+$ perivascular stromal cells	Inhibits protein tyrosine phosphatase receptor-ζ	HSCs	Knockout mice have a reduced HSC frequency and show impaired recovery after irradiation	Dispensable for steady-state haematopoiesis but seems to promote HSC maintenance
SLIT ligands[177–179]	SLIT2 and SLIT3 are produced by perivascular stromal cells	ROBO4	HSCs and sinusoidal endothelial cells	Has cell-autonomous and non-cell-autonomous effects on HSC engraftment and mobilization	Dispensable for haematopoiesis, but *Robo4*$^{-/-}$ mice have HSC depletion, sinusoid defects and increased vascular permeability
TGFβ[27]	Multiple cells including megakaryocytes; other cells such as Schwann cells regulate TGFβ activation[109]	Multiple type I and type II TGFβ receptors	HSCs and other cell types	Promotes HSC quiescence and self-renewal	With multiple ligands and receptors, the genetics of the TGFβ family are complex, and genetic manipulation leads to a range of different phenotypes
WNT ligands[180,181]	Bone marrow stromal cells and lymphoid cells	Frizzled receptors	HSCs and lymphoid progenitors	Canonical WNT signalling through β-catenin and γ-catenin is dispensable for adult HSC maintenance and for haematopoiesis	Canonical WNT signalling is not required for normal adult haematopoiesis; non-canonical WNT signalling remains under investigation[182]

BMP, bone morphogenetic protein; FGF, fibroblast growth factor; FGFR, FGF receptor; G-CSF, granulocyte colony-stimulating factor; HSC, haematopoietic stem cell; IL-6, interleukin-6; LEPR, leptin receptor; LSK, lineage$^-$SCA1$^+$KIT$^+$; ROBO4, roundabout guidance receptor 4; SCA1, spinocerebellar ataxia type 1 protein; TGFβ, transforming growth factor-β; TIE2, tyrosine kinase with Ig and EGF homology domains 2.

regeneration after injury; these growth factors include angiogenin[64], angiopoietin-like protein 3 (REF. 65), fibroblast growth factor 1 (FGF1)[27,66], FGF2 (REF. 67), interleukin-6 (IL-6)[68], Notch 2 (REF. 69) and pleiotrophin[70]. It is not yet clear to what extent these factors are synthesized by HSC niche cells versus other cell types in the bone marrow. The isolation and characterization of individual stromal cells adjacent to engrafting HSCs may help to identify new factors that regulate haematopoietic regeneration[71]. Understanding how the niche changes in response to injury is another important area for future research.

Long-range factors that are synthesized outside the bone marrow at sites distant from the HSC niche also modulate HSC or niche function, sometimes in response to physiological changes (TABLE 3).

In addition to ligand–receptor interactions, it is likely that there are metabolic and physical features of the microenvironment that also promote HSC maintenance. Evidence suggests that most HSCs reside in a relatively hypoxic region of the bone marrow[72] and that perisinusoidal microenvironments have the lowest oxygen tension in the bone marrow[73]. HSCs also seem to be more sensitive to valine depletion than to the depletion of other amino acids[74]. An important question for the future is whether the niche regulates nutrient availability in a manner that is necessary for HSC maintenance. If so, this might begin to explain why no known combination of growth factors is sufficient to sustain HSCs in culture.

Identifying the cells that make niche factors

CXCL12 was the first HSC niche factor for which expression was systematically characterized in the bone marrow[12,75,76]. These studies used immunostaining and *Cxcl12* knock-in reporter mice to conclude that CXCL12 is mainly expressed by perivascular stromal cells and endothelial cells that are associated with sinusoids throughout the bone marrow, and that lower levels of CXCL12 are expressed by osteoblasts[12,77]. The perivascular stromal cells were described as CAR cells by Nagasawa and colleagues[12]. Relative to these perivascular cells, endothelial cells and osteoblasts express *Cxcl12* at approximately 100-fold and 1,000-fold lower levels, respectively[78]. CXCL12 is also expressed by stromal cells localized around arterioles[78,79], although such blood vessels are much less numerous than are sinusoids in the bone marrow (FIG. 1).

The perivascular stromal cells and endothelial cells that synthesize CXCL12 are the same cells that are the major sources of SCF in the bone marrow[78]. *Scf* is mainly expressed by perivascular stromal cells that are associated with sinusoidal blood vessels throughout the bone marrow and is expressed at a much lower level by endothelial cells. Similarly to *Cxcl12*, *Scf* is also expressed around small-diameter arterioles[17] (FIG. 2). *Scf* expression is not detectable in osteoblasts, megakaryocytes or other haematopoietic cells[17]. Thus, perivascular stromal cells and endothelial cells produce multiple factors that are crucial components of the HSC niche.

Ng2-CreER$^+$ cells
Cells that express a tamoxifen-activated form of Cre recombinase from the *Ng2* locus (which encodes neural–glial antigen 2). The conditional reporter is expressed by rare periarteriolar stromal cells in adult bone marrow but is much more widely expressed in early postnatal bone marrow.

The vast majority of the perivascular stromal cells that express *Scf* and *Cxcl12* can be identified on the basis of their expression of the full-length leptin receptor (LEPR)[17]: 90–95% of cells that express high levels of *Scf*-GFP or *Cxcl12*-DsRed in young-adult bone marrow are LEPR$^+$ cells[24]. In normal young-adult bone marrow, there is a nearly complete overlap between cells that express high levels of *Cxcl12* (CAR cells) and LEPR$^+$ cells[24]. The LEPR$^+$ cells therefore seem to be the same cells that were described as CAR cells[12,17,23,24,78] (TABLE 1). Consistent with this, the conditional deletion of *Foxc1* — which encodes forkhead box protein C1, a transcription factor that is required by CAR cells — using *Lepr*-Cre substantially depletes HSCs and reduces bone marrow cellularity[80].

LEPR$^+$/CAR cells represent approximately 0.3% of bone marrow cells, but they have long processes that are present throughout the bone marrow. HSCs are closely associated with LEPR$^+$ cells[14,17,24,78] and CAR cells[12,80]. LEPR$^+$ cells that express *Scf* and *Cxcl12* are associated with both sinusoids and small-diameter arterioles, and therefore could potentially contribute to perivascular niches that are associated with both types of blood vessel (FIGS 2,3).

Scf and *Cxcl12* are also expressed by *Nes*-GFP$^+$ bone marrow stromal cells[13,25]. These *Nes*-GFP$^+$ cells include both *Nes*-GFP^{low} cells that are perisinusoidal and LEPR$^+$ (REFS 17,81), as well as rare *Nes*-GFP^{hi} cells that are periarteriolar, *Nes*-CreER$^+$ (REF. 25) and *Ng2*-CreER$^+$ (REF. 13) (TABLE 1). Thus, it is not clear whether the expression of *Scf* and *Cxcl12* by *Nes*-GFP$^+$ cells reflects expression by perisinusoidal *Nes*-GFP^{low}LEPR$^+$ cells or expression by periarteriolar *Nes*-GFP^{hi}*Nes*-CreER$^+$ cells. Consistent with the former possibility, RNA sequencing of *Nes*-GFP$^+$ cells showed that the cells that express high levels of *Scf* and *Cxcl12* are positive for *Lepr* expression and negative for endogenous *Nes* expression[13]. Results from studies in which *Scf* and *Cxcl12* have been conditionally deleted are also equivocal with respect to the existence of a distinct periarteriolar niche cell (see the next section). It will be important in future studies to determine whether any of the *Scf* and *Cxcl12* expressed by *Nes*-GFP$^+$ stromal cells reflects expression by periarteriolar niche cells that are distinct from LEPR$^+$/CAR cells. These questions are also complicated by the fact that all of the *Nes* reporter alleles are transgenes that have different genomic integration sites and different expression patterns[17,82]. Moreover, cells that express *Nes* transgenes do not necessarily express endogenous *Nes*[82]. Therefore, although some studies have interchangeably used nestin-specific antibodies and various *Nes* transgenes as stromal cell markers, these transgenes often have quite different expression patterns in the bone marrow[17], and it remains unclear whether any of the cells that are marked by *Nes* transgene expression express endogenous nestin.

Conditional deletion of niche factors

Niche cells can be functionally identified by conditionally deleting known niche factors from candidate cell populations and then testing for effects on stem cell maintenance. The conditional deletion of

Table 3 | **Examples of long-range factors that regulate adult HSCs or the HSC niche**

Factor	Source	Receptor	Responding cell type or types	Effect on HSCs or niche	Physiological role
Oestrogen[121]	Ovary	ERα	HSCs as well as other haematopoietic cells	Promotes HSC self-renewal and EMH	Expansion of splenic erythropoiesis during pregnancy
IGF1 (REFS 183,184)	Liver and osteoblasts	IGF1R	HSCs, myeloid progenitors and pro-B cells	Reduced plasma IGF1 levels promote increased HSC self-renewal and increase the frequency of myeloid progenitors	Prolonged fasting reduces IGF1 levels and improves haematopoietic recovery after myeloablation
Leptin[163]	Adipocytes	LEPR	LEPR$^+$/CAR perivascular stromal cells	Causes LEPR$^+$ cells to form adipocytes at the expense of bone[24]	Nutritional regulation of SSC fate; dispensable for haematopoiesis
Thrombopoietin	Highest production in the liver and kidney, but the source of the thrombopoietin that acts on HSCs is not known[60,63]	MPL	HSCs and megakaryocytes	Required for the postnatal maintenance of HSCs[38–41], and for megakaryocyte and platelet production[60–62]	HSC maintenance and thrombopoiesis

CAR cell, CXCL12-abundant reticular cell; CXCL12, CXC-chemokine ligand 12; EMH, extramedullary haematopoiesis; ERα, oestrogen receptor-α; HSC, haematopoietic stem cell; IGF1, insulin-like growth factor 1; IGF1R, IGF1 receptor; LEPR, leptin receptor; MPL, myeloproliferative leukaemia protein; SSC, skeletal stem cell.

Scf from haematopoietic cells, megakaryocytes, osteoblasts, *Nes*-Cre$^+$ cells, *Nes*-CreER$^+$ cells or *Ng2*-CreER$^+$ cells does not significantly affect HSC frequency, HSC function or haematopoiesis in adult bone marrow[14,17,24,78] (TABLE 4). This is consistent with the observation that none of these cell populations detectably expresses an *Scf*-GFP knock-in reporter[17]. By contrast, when *Scf* is conditionally deleted from endothelial cells or LEPR$^+$ cells, HSCs are depleted from the bone marrow without any apparent effect on stromal cell frequency or blood vessel morphology[17,79] (TABLE 4). When *Scf* is deleted from both endothelial cells and LEPR$^+$ cells in *Tie2*-Cre;*Lepr*-Cre;*Scf* $^{fl/-}$ mice, HSCs are substantially depleted from adult bone marrow and there is a loss of all quiescent and serially transplantable HSCs[83] (TABLE 4). The HSC niche in adult bone marrow thus depends on SCF that is produced by endothelial cells and LEPR$^+$ stromal cells. Other haematopoietic progenitors also depend on SCF and may also be depleted as a result of *Scf* deletion from LEPR$^+$ cells.

The same endothelial cells and LEPR$^+$ cells are also functionally important sources of the CXCL12 required for HSC maintenance in adult bone marrow. The conditional deletion of *Cxcl12* from haematopoietic cells, megakaryocytes, osteoblasts, *Nes*-Cre$^+$ cells, *Nes*-CreER$^+$ cells or *Ng2*-CreER$^+$ cells in adult bone marrow does not significantly affect HSC frequency or HSC function[14,24,78,84] (TABLE 4). The deletion of *Cxcl12* from endothelial cells or paired-related homeobox 1 (PRRX1)-expressing mesenchymal stromal cells (TABLE 1) depletes HSCs from adult bone marrow with no apparent effect on stromal cell frequency or blood vessel morphology[78,84] (TABLE 4). The conditional deletion of *Cxcl12* using *Lepr*-Cre mobilizes HSCs from the bone marrow without detectable HSC depletion[78,79]. LEPR$^+$ cells are thus an important source of the CXCL12 that is required for the retention of HSCs in the bone marrow, although HSC depletion is more severe when *Cxcl12* is conditionally deleted using *Prrx1*-Cre than when using *Lepr*-Cre. This is probably because PRRX1 is more broadly expressed by bone marrow stromal cells than is LEPR: *Prrx1*-Cre recombines in osteoblasts and other stromal cells in addition to LEPR$^+$ cells[78,84].

HSCs had previously been proposed to be maintained in osteoblastic (endosteal) niches, but the available evidence no longer supports this model[9] as osteoblasts are not a necessary source of any niche factor that is known to be required for HSC maintenance. The conditional deletion of *Cxcl12* from osteoblasts does not affect HSC frequency or function, but instead depletes a subset of early lymphoid progenitors[78,84]. This is consistent with the observation that diphtheria toxin-mediated ablation of osteoblasts acutely depletes lymphoid progenitors but not HSCs[21,22] (TABLE 4). This osteoblastic niche for lymphoid progenitors seems, however, to support the maintenance of only a subset of these progenitors, as most stages of B cell development occur in perisinusoidal niches in which CXCL12 and IL-7 are synthesized by cells that express osterix (also known as SP7) and LEPR[23,84–88].

Sinusoidal versus arteriolar niches

It has also been proposed that there is a periarteriolar niche for HSCs that is distinct from the perivascular niche created by LEPR$^+$ cells[13]. This model was based initially on the suggestion that periarteriolar *Nes*-CreER$^+$ and *Ng2*-CreER$^+$ cells are an important source of SCF and CXCL12 in the bone marrow[13,79]. However, it now seems clear that these periarteriolar cells are not a source of the SCF required for HSC maintenance, as no *Scf*-GFP expression can be detected in these cells[14,17] and the conditional deletion of *Scf* using *Nes*-Cre[17], *Nes*-CreER[17] or *Ng2*-CreER[14,79] has no effect on HSC frequency or haematopoiesis in adult bone marrow.

The data with respect to periarteriolar CXCL12 expression are less clear. Low levels of staining with CXCL12-specific antibody were observed by flow cytometry in fixed *Ng2*-CreER$^+$ bone marrow cells[79].

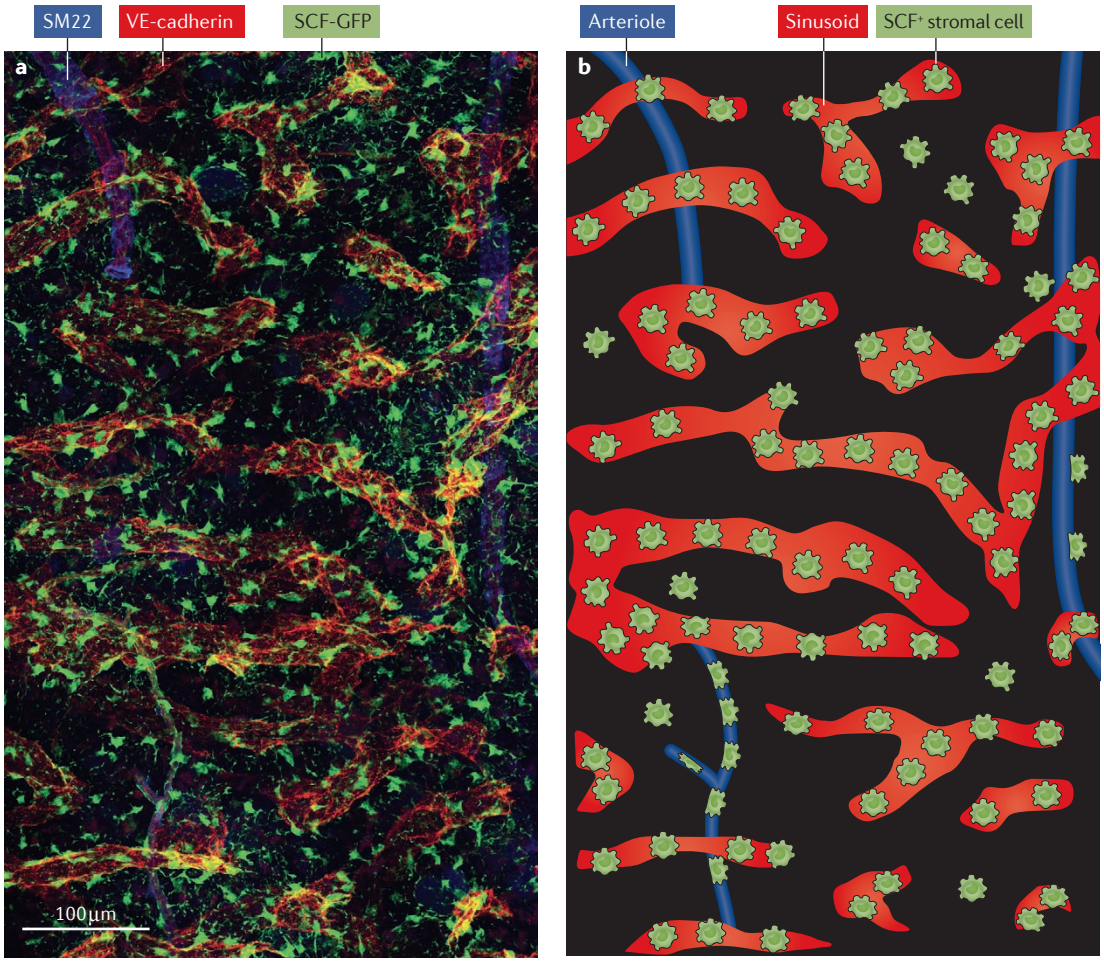


Figure 2 | **The expression of stem cell factor in the bone marrow.** Of the known niche factors, stem cell factor (SCF) is one of the most important in terms of the maintenance of haematopoietic stem cells (HSCs)[16,33,34,52,53]. **a** | A 3D reconstruction of a thick bone marrow specimen from an *Scf*$^{GFP/+}$ mouse that was stained with antibodies specific for green fluorescent protein (GFP) to identify *Scf*-expressing cells (green), vascular endothelial cadherin (VE-cadherin) to identify endothelial cells (red) and smooth muscle protein 22 (SM22; also known as transgelin) to identify smooth muscle cells associated with arterioles (blue). **b** | A schematic representation of the image shown in part **a**. SCF is expressed mainly by perivascular stromal cells that are associated with sinusoids throughout the bone marrow. These SCF$^+$ stromal cells also express high levels of CXC-chemokine ligand 12 (CXCL12)[23,24] and leptin receptor[17,24,78,79]. Smaller numbers of SCF$^+$ cells are associated with small-diameter arterioles. Endothelial cells express SCF and CXCL12 at much lower levels, which cannot be appreciated in this image. Part **a** is reproduced with permission from REF. 24, Elsevier.

However, no *Cxcl12*-DsRed or *Cxcl12*-GFP knock-in reporter expression could be detected in cells that recombined with either *Ng2*-CreER or *Nes*-CreER[14,17,24,78,79]. The deletion of *Cxcl12* using *Ng2*-CreER or *Nes*-CreER depletes HSCs when tamoxifen is administered within the first 3 weeks after birth[79,89], when these Cre alleles are broadly expressed in the bone marrow[32]; however, HSCs are not depleted when tamoxifen is administered at 6 weeks after birth, after the expression of *Ng2*-CreER and *Nes*-CreER becomes more restricted to periarteriolar cells[14,78]. The conditional deletion of *Cxcl12* using *Ng2*-Cre depletes bone marrow HSCs to a greater extent than does *Cxcl12* deletion using *Lepr*-Cre[79]; however, *Ng2*-Cre is expressed much more widely than is *Lepr*-Cre in perinatal bone marrow, leading to a severe developmental depletion of HSCs in neonatal bone marrow that is not observed with *Lepr*-Cre (M. M. Murphy and S.J.M., unpublished observations). The simplest interpretation that takes into account all of the data is that NG2$^+$ cells are a developmentally important source of the CXCL12 required for the colonization of the bone marrow by HSCs around the time of birth, when NG2 expression in the bone marrow is widespread and not limited to periarteriolar cells. By adulthood, when NG2$^+$ cells in the bone marrow are rare, the data do not presently support the conclusion that periarteriolar *Ng2*-CreER$^+$ cells are an important source of the CXCL12 required for HSC maintenance.

Irrespective of whether NG2$^+$nestin^{hi} periarteriolar cells are a source of the factors required for HSC maintenance, approximately 10% of HSCs are more closely associated with arterioles than with sinusoidal blood vessels[13,14,90,91], which raises the question of whether arterioles and sinusoids create functionally

distinct perivascular niches. The periarteriolar and perisinusoidal microenvironments differ with respect to oxygen tension[73,90], vessel wall permeability[90] and the capacity of haematopoietic cells to migrate through the vessel wall[90,92]. The question is whether these differences affect the properties of the HSCs that localize to these microenvironments.

It has been proposed that HSCs associated with arterioles are more quiescent than are those associated with sinusoids[13]; however, there is not yet any functional evidence that the arteriolar microenvironment is better at promoting quiescence than is the perisinusoidal microenvironment. All of the markers that have been used to localize HSCs around sinusoids identify overwhelmingly quiescent cell populations[10,14]. Therefore, the direct evidence continues to suggest that the majority of quiescent HSCs are most closely associated with sinusoids, while leaving open the possibility that a subset of quiescent HSCs is associated with arterioles.

Some differences have been observed between the periarteriolar and perisinusoidal microenvironments. $CD150^{+}CD48^{-}$ cells (which are highly enriched for HSCs[10]) adjacent to arterioles stain negatively for reactive oxygen species (ROS), whereas 36% of $CD150^{+}CD48^{-}$ cells adjacent to sinusoids stain positively for ROS[90]. This may reflect increased ROS levels in perisinusoidal HSCs

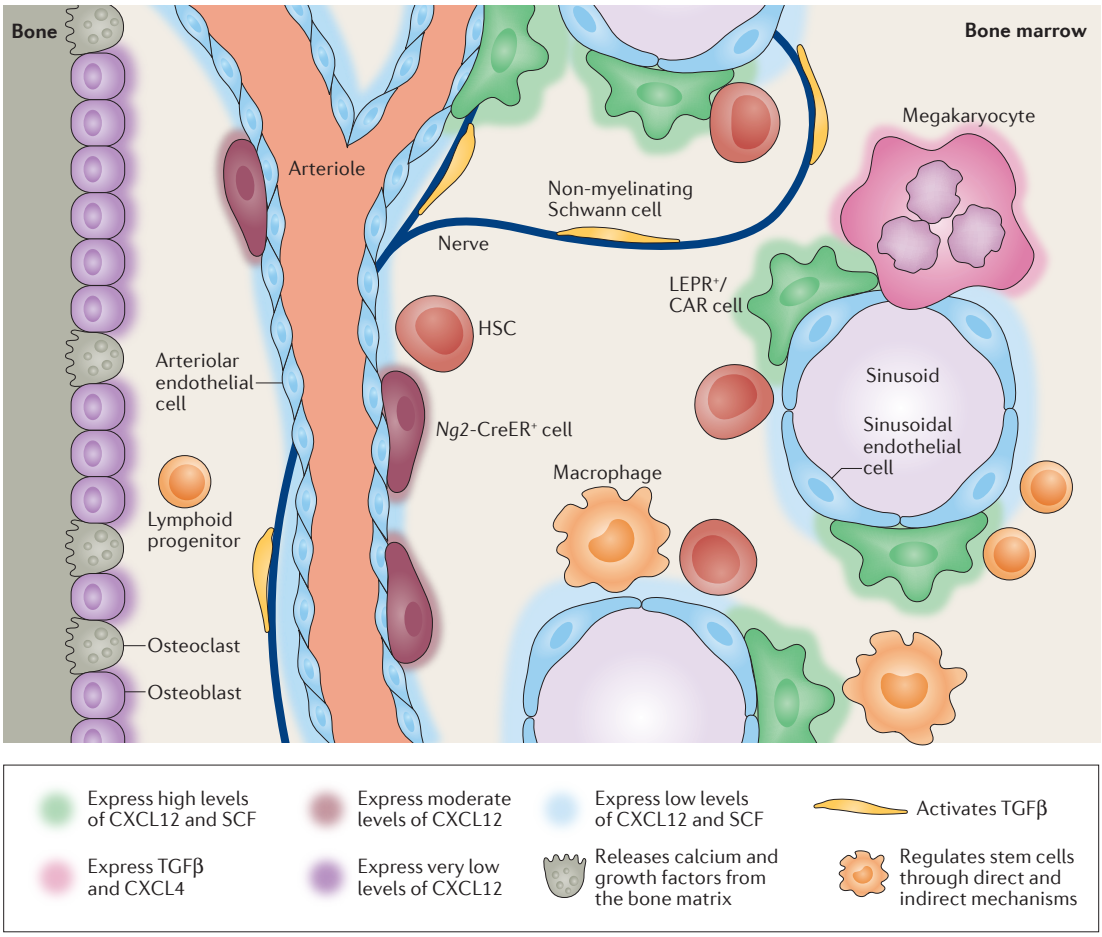


Figure 3 | **A schematic of the HSC niche in adult bone marrow.** Most haematopoietic stem cells (HSCs) localize adjacent to sinusoids, where they are in close contact with leptin receptor (LEPR)-expressing mesenchymal stromal cells (also known as CXC-chemokine ligand 12 (CXCL12)-abundant reticular (CAR) cells) and endothelial cells, both of which are necessary sources of the stem cell factor (SCF) and CXCL12 required for HSC maintenance[10–14]. Approximately 10% of HSCs localize near to small-diameter arterioles[14], which are also associated with $LEPR^{+}$ stromal cells, as well as rare *Ng2*-$CreER^{+}$ cells[13] (in which a tamoxifen-activated form of Cre recombinase is expressed from the *Ng2* locus, which encodes neural–glial antigen 2) that may[13,79] or may not[14] be a source of the CXCL12 required for HSC maintenance. Nerve fibres[107], Schwann cells associated with nerve fibres[109], megakaryocytes[26,27], macrophages[28–30,108,111] and osteoclasts[112–114] also regulate HSC maintenance through several mechanisms. Osteoblasts do not directly regulate HSC maintenance through any known mechanism[9], but they probably indirectly regulate HSC maintenance through crosstalk with other cell types in the bone marrow, such as the cells comprising the vasculature[91,96]. Osteoblasts promote the maintenance of a subset of early lymphoid progenitors by secreting very low levels of CXCL12 (REF. 78), but other lymphoid progenitors reside in sinusoidal niches, where they depend on CXCL12 synthesized by $LEPR^{+}$ cells[85,86]. TGFβ, transforming growth factor-β.

Table 4 | **Conditional deletion of *Scf* and *Cxcl12* to determine their cellular sources for HSCs in the bone marrow**

Cell type	Cre allele	Effect of *Scf* deletion	Effect of *Cxcl12* deletion
Endothelial cells and LEPR$^+$ stromal cells	*Tie2*-Cre and *Lepr*-Cre	Loss of all quiescent and serially transplantable HSCs from the bone marrow[17,83]	ND
Endothelial cells	*Tie2*-Cre	HSC depletion[17]	HSC depletion[78,84]
Haematopoietic cells	*Vav1*-Cre	No effect[17]	No effect[78]
Perisinusoidal stromal cells	*Lepr*-Cre	HSC depletion[17,79]	HSC mobilization[78,79]
	Il7-Cre	HSC and multipotent progenitor depletion[86]	HSC and B cell progenitor depletion[86]
Osteoblasts	*Col2.3*-Cre	No effect[17]	Lymphoid progenitor depletion[78]
	Ocn-Cre	ND	Lymphoid progenitor depletion[84]
Osterix$^+$ stromal cells	*Osx*-Cre	ND	HSC mobilization and lymphoid progenitor depletion[84]
Periarteriolar stromal cells	*Nes*-Cre	No effect[17]	No effect[78]
	Nes-CreER	No effect of deletion at 8 weeks of age[17]	No effect in adult bone marrow[78]; HSC depletion in early postnatal bone marrow[89]
	Ng2-Cre	HSC depletion[79]	HSC and committed progenitor depletion[79]
	Ng2-CreER	No effect of deletion at 2–3 weeks[79] or 6 weeks[14] of age	HSC mobilization when deleted at 2–3 weeks of age[79] but no effect when deleted at 6 weeks of age[14]
PRRX1$^+$ stromal cells	*Prrx1*-Cre	ND	HSC mobilization and depletion, and lymphoid progenitor depletion[78,84]
Ubiquitous	*Ubc*-CreER	HSC depletion when deleted at 8 weeks of age[17]	HSC depletion when deleted at 8 weeks of age[78]
	CMV-Cre	Embryonic lethal[17]	Embryonic lethal[78]
	E2a-Cre and *TgCAGG*-CreER	ND	HSC mobilization, and HSC and lymphoid progenitor depletion when deleted at 10 weeks of age[57]

Col2.3, 2.3 kb fragment of rat *Col1a1*; CreER, tamoxifen-activated form of Cre recombinase; *Cxcl12*, CXC-chemokine ligand 12; *CMV*, human cytomegalovirus immediate early enhancer and promoter; HSC, haematopoietic stem cell; *Il7*, interleukin-7; *Lepr*, leptin receptor; ND, not determined (indicates that the experiment has not been carried out to our knowledge); *Nes*, nestin; *Ng2*, neural–glial antigen 2; *Ocn*, osteocalcin; *Osx*, osterix; *Prrx1*, paired-related homeobox 1; *Scf*, stem cell factor; *TgCAGG*-CreER, tamoxifen-inducible Cre recombinase under the control of the ubiquitous *CMV–Actb* (β-actin) promoter; *Tie2*, tyrosine kinase with Ig and EGF homology domains 2; *Ubc*, ubiquitin C; *Vav1*, vav guanine nucleotide exchange factor 1.

compared with periarteriolar HSCs. However, 37% of CD150$^+$CD48$^-$ bone marrow cells are CD41$^+$ megakaryocyte progenitors that lack HSC activity[10], and these cells are likely to associate with sinusoids[93]. Thus, it is unclear whether the difference in ROS staining reflects heterogeneity among HSCs, or heterogeneity between HSCs and megakaryocyte progenitors.

Sinusoidal blood vessels have a fenestrated basal lamina and are the site of blood cell migration between the bone marrow and the circulation. As such, they are more leaky than are arterioles, and this potentially increases the exposure of perisinusoidal HSCs to blood plasma components and increases ROS levels[90]. Consistent with this, genetic changes that increase vascular permeability *in vivo* increase ROS levels in CD150$^+$CD48$^-$ cells, as well as their mobilization and apoptosis[90]. However, CD150$^+$CD48$^-$ cells from mutant mice with increased vascular permeability did not exhibit significant changes in cell cycle status, and they had an increased capacity to reconstitute the myeloid lineage in irradiated mice[90]; this property is associated with more-quiescent HSCs[94]. These data argue against the idea that increased vascular permeability decreases HSC quiescence. What is clear is that HSCs enter and exit the circulation through sinusoids[90], and that their sinusoidal localization therefore facilitates their ability to traffic through the blood[95].

Increased Notch signalling in endothelial cells increases the number of arterioles in the bone marrow, as well as SCF expression and HSC frequency, and reduced Notch signalling decreases the number of arterioles, SCF expression and HSC frequency[96]. This raises the possibility that HSC frequency in the bone marrow is directly or indirectly determined by arteriole density, although the genetic approach that was used to modulate Notch signalling in the experiments that provided these findings (that is, expression of tamoxifen-activated Cre recombinase under the control of the promoter of *Cdh5*, which encodes vascular endothelial cadherin (*Cdh5*-CreER)) would be expected to affect sinusoidal endothelial cells as well as arteriolar endothelial cells. Therefore, it is uncertain to what extent the changes in HSC frequency reflected changes in Notch signalling in arterioles versus sinusoidal blood vessels in these experiments.

Sinusoids are severely damaged by irradiation[97], whereas arterioles seem to be more resilient and may give rise to the endothelial cells that regenerate sinusoids after irradiation[96]. Thus, HSCs might become more dependent on periarteriolar niches during the regeneration of haematopoiesis after irradiation, and arterioles may be important for the regeneration of normal vasculature in the bone marrow.

Skeletal stem cells and the HSC niche

Skeletal stem cells (SSCs) form fibroblast colonies in culture (colony-forming unit-fibroblasts (CFU-Fs)) and can undergo multilineage differentiation into osteoblasts, chondrocytes and adipocytes[98]. SSCs are maintained throughout life in the bone marrow, and give rise to osteoblasts that maintain the skeleton, as well as the adipocytes that accumulate with age in the bone marrow. SSCs may regulate the formation of HSC niches during development[99], and they are among the stromal cells that secrete HSC niche factors in adult bone marrow[17,23,25,84,100]. The first evidence for this came from the observation that $CD146^+$ SSCs from human bone marrow give rise to bony ossicles after transplantation into immunocompromised mice that become colonized with endogenous HSCs and that support haematopoiesis[100]. The $CD146^+$ cells localize around sinusoids in the ossicles and synthesize factors that are associated with the HSC niche, such as angiopoietin 1 (REF. 100), which regulates niche regeneration after injury[101]. In mice, bone marrow stromal cells that express the *Nes*-GFP transgene, *Scf* or *Cxcl12* are highly enriched for CFU-Fs that differentiate in culture to form osteoblasts, chondrocytes and adipocytes[23–25]. Consistent with this, bone marrow stromal cells that express *Lepr*-Cre are also highly enriched for CFU-Fs, and give rise to most of the osteoblasts and adipocytes that form in adult bone marrow[24,102].

Although the stromal cells that express HSC niche factors are highly enriched for SSCs, this cell population is heterogeneous, and only a subset of the cells are SSCs (perhaps 10%). Consequently, it is not clear whether SSCs colocalize with HSCs or whether SSCs preferentially localize to a distinct region of bone marrow, such as the metaphysis[103,104], where they are not necessarily associated with HSCs. More markers are required to resolve the heterogeneity among bone marrow stromal cells.

Niche regulation by other cell types

Multiple cell types directly or indirectly regulate HSC maintenance through mechanisms other than SCF or CXCL12 synthesis.

Megakaryocytes. Megakaryocytes localize to sinusoids[93] and are often closely associated with HSCs[27]. The ablation of megakaryocytes leads to increased HSC proliferation and HSC frequency[26,27], although it is unclear to what extent this reflects an injury response to compensate for thrombocytopenia versus a direct inhibition of HSC cell division by megakaryocytes. In support of the latter possibility, megakaryocytes produce transforming growth factor β1 (TGFβ1; encoded by *Tgfb1*)[27], which promotes HSC quiescence *in vivo*[105]. Conditional deletion of *Tgfb1* from megakaryocytes increases HSC proliferation *in vivo*[27]. Megakaryocytes are also a source of CXCL4 (also known as platelet factor 4) and *Cxcl4*-deficient mice have increased HSC numbers and HSC proliferation. The administration of recombinant CXCL4 (REF. 26) or TGFβ1 (REF. 27) after megakaryocyte ablation reduces the HSC proliferation that would otherwise occur.

Megakaryocytes can also promote the regeneration of HSCs after myeloablation by synthesizing FGF1 (REF. 27), a cytokine that acts on HSCs to promote regeneration after injury[66]. Thus, there are multiple mechanisms by which megakaryocytes seem to regulate HSC function.

Nerve cells. Nerve fibres, and the Schwann cells associated with them, also regulate HSC function through multiple mechanisms. Nerve fibres are not required for the maintenance of HSCs in the bone marrow[106,107], although they are required for the regeneration of haematopoiesis after chemotherapy[106]. Nerve fibres also regulate the daily circadian rhythm of HSC mobilization from the bone marrow into the blood, perhaps by regulating the cyclical expression of CXCL12 by stromal cells[107]. Circadian CXCL12 expression and HSC mobilization are also regulated by the clearance of aged neutrophils from the circulation and their subsequent engulfment by macrophages[108], which further highlights the complexity of interactions between cell types in the bone marrow. Finally, non-myelinating Schwann cells regulate the proteolytic activation of latent TGFβ in the bone marrow[109], and TGFβ promotes HSC maintenance.

Most nerve fibres are closely associated with arterioles[13,90] in the bone marrow, whereas most HSCs are associated with sinusoids[10–13] and do not localize near nerve fibres[14]. Therefore, most HSC niche cells are not directly innervated. However, efferent nerve terminals do synapse on a subset of perivascular stromal cells that are associated with sinusoids, and networks of these perisinusoidal stromal cells are inter-connected by gap junctions[110]. Thus, HSCs and niche cells may be regulated by signals that are transmitted through these networks from nerve fibres, or they may be otherwise influenced by diffusible factors produced by Schwann cells[109] or nerve fibres. Nonetheless, little is known about the nature of these signals or the mechanisms by which they might propagate through stromal cell networks.

Monocytes and macrophages. The depletion of bone marrow macrophages reduces CXCL12 expression in the bone marrow and promotes HSC mobilization[28,29]. A subset of macrophages that express Duffy antigen receptor for chemokines (DARC; also known as CD234 and ACKR1) may regulate TGFβ–SMAD3 signalling in HSCs[30]. A rare subset of smooth muscle actin (SMA)-expressing macrophages express cyclooxygenase 2 (COX2) and are adjacent to HSCs in the bone marrow[111]. These cells expand in number following the administration of lipopolysaccharide and produce prostaglandin E2, and this expansion correlates with decreased ROS levels in HSCs. In addition, osteoclasts — which derive from the monocyte lineage — may regulate HSC function through their effects on bone remodelling, by modulating local Ca^{2+} concentrations, by releasing factors from the bone matrix and by producing cytokines[112–114].

Stromal cell changes with age

The stromal cell composition of the bone marrow changes over time between the early postnatal period and adulthood. Multiple waves of mesenchymal stem

Skeletal stem cells
(SSCs). A rare self-renewing stem cell population in the bone marrow that has the potential to form osteoblasts, adipocytes and chondrocytes, and that has the physiological function of maintaining the adult skeleton.

Schwann cells
Neural crest-derived cells that include myelinating and non-myelinating glia that are associated with peripheral nerve fibres in the bone marrow.

and progenitor cells colonize the bone marrow during development, thus altering the composition of the bone marrow stroma over time. During late fetal development, SSCs marked by the expression of *Osx* (which encodes osterix)[102,115], *Col2a1* (which encodes collagen type II α1 chain)[116] and *Sox9* (which encodes SRY-box 9)[116] give rise to bone and bone marrow stromal cells that persist postnatally. However, many of these stromal cells are depleted by adulthood[102,117], and these markers do not seem to label SSCs in adult bone marrow[102,115–117]. Neural crest-derived cells marked by *P0*-Cre (*P0*, also known as *Rplp0*, encodes protein 0), *Sox1*-Cre or *Wnt1*-Cre make a transient contribution to bone marrow stromal cells and CFU-F activity in early postnatal bone marrow, but these neural crest-derived cells are also depleted by adulthood and are replaced by non-neural-crest-derived stromal cells[89,118,119]. Consistent with this, *Nes*-CreER$^+$ stromal cells contribute to osteogenesis in early postnatal bone marrow[32], but make little or no contribution to CFU-F activity or to osteogenesis in adult bone marrow[24,32]. Conversely, LEPR$^+$ cells arise postnatally in the bone marrow; they make little contribution to the stromal cell population or to osteogenesis in early postnatal bone marrow, but they expand in number over time and are the main source of osteoblasts and adipocytes in adult bone marrow[24].

Consistent with these changes in the stroma, the cellular composition of the HSC niche also changes over time. The depletion of *Nes*-CreER$^+$ cells using diphtheria toxin at embryonic day 15.5 impairs the colonization of fetal bone marrow by HSCs, and the conditional deletion of *Cxcl12* using *Nes*-CreER at postnatal day 7 depletes bone marrow HSCs[89]. By contrast, the conditional deletion of *Cxcl12* or *Scf* using *Nes*-CreER in adult mice has no effect on HSC frequency or haematopoiesis[17,78]. Consistent with this, *Nes*-CreER$^+$ stromal cells are abundant in early postnatal bone marrow[32] but very rare in adult bone marrow[17,24]. *Nes*-CreER$^+$ cells thus seem to be a component of the HSC niche in early postnatal, but not adult, bone marrow.

There are also changes in the bone marrow vasculature and bone marrow stroma during ageing that can be at least partially reversed by the activation of Notch signalling in endothelial cells[96] (FIG. 4). These results raise the possibility that some of the changes observed in HSCs during ageing reflect changes in the vasculature or the stromal composition of the niche. Understanding the differences between the early postnatal period, young adulthood and old adulthood will be an important focus for future studies.

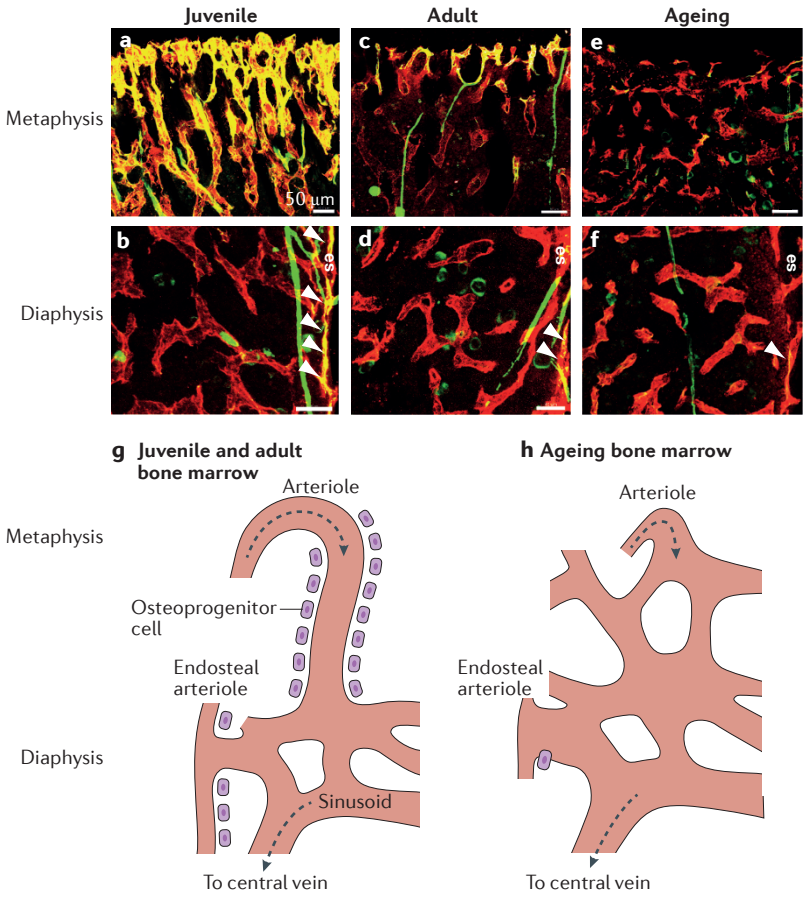


Figure 4 | **Changes in the bone marrow vasculature with age.** Confocal images of metaphyseal and diaphysial portions of tibia taken from juvenile mice (parts **a** and **b**; 4 weeks of age), adult mice (parts **c** and **d**; 11 weeks of age) and ageing mice (parts **e** and **f**; 70 weeks of age), and stained with antibodies specific for CD31 (green) and endomucin (red). Note the age-related decline in the frequency of arterioles expressing high levels of both CD31 and endomucin (yellow), which are predominantly localized in the metaphysis and adjacent to the endosteum (labelled 'es' and indicated by white arrowheads in parts **b**, **d** and **f**)[91,96]. These endosteal arterioles overlap with the transition zone vessels shown in FIG. 1. These vessels are located near osteprogenitors, as illustrated schematically in parts **g** and **h**, in which dashed arrows indicate the direction of blood flow. By contrast, sinusoidal vessels, which express lower levels of CD31 and endomucin, remain stable in frequency and distribution during ageing. Reproduced with permission from REF. 96, Macmillan Publishers Limited.

The extramedullary niche

Whereas changes in the stromal composition and vascular structure of the niche may affect HSC function during ageing, more abrupt changes in the HSC microenvironment occur during times of haematopoietic stress when HSCs are mobilized and colonize facultative niches in extramedullary tissues. This may occur in the setting of pregnancy[120,121], anaemia, other haematological disorders or infection[122–124]. The most common sites for extramedullary haematopoiesis (EMH) are the spleen and liver, but EMH can occur in almost any tissue[122]. The niche for EMH has been characterized in the spleen, where EMH and HSCs are sustained by perivascular niches that are associated with sinusoidal blood vessels in the red pulp[10,120]. Conditional deletion studies show that splenic EMH depends on SCF synthesized by endothelial cells and transcription factor 21 (TCF21)-expressing perivascular stromal cells, as well as CXCL12 synthesized by a subset of TCF21$^+$ stromal cells[120]. This demonstrates that, like in the bone marrow, HSCs are mainly perisinusoidal in the spleen, and they depend on SCF and CXCL12 synthesized by endothelial cells and perivascular stromal cells. However, the niche cells

in the spleen express different markers from those expressed by the niche cells in the bone marrow: perivascular stromal cells are TCF21$^+$ and LEPR$^-$ in the spleen but TCF21$^-$ and LEPR$^+$ in the bone marrow[120].

This splenic EMH niche is necessary for the recovery of normal blood cell counts after various haematopoietic stresses in mice, including pregnancy, bleeding and myeloablation[120]. In humans, EMH also seems to occur preferentially in the liver and spleen, and EMH in these tissues may also be necessary for recovery from haematopoietic stresses; patients who have EMH in other tissues have frequently undergone a prior splenectomy, and many of these patients depend on erythrocyte transfusions[125].

An important unresolved issue is the identity of the signals that induce EMH in response to haematopoietic stress. Some evidence suggests that HSCs migrate to extramedullary tissues as a result of a hypoxia-inducible factor 1 (HIF1)-mediated increase in CXCL12 expression in endothelial cells in response to hypoxia[126], as can occur in the heart after myocardial infarction[127]. However, SCF$^+$ endothelial cells and CXCL12$^+$ perivascular stromal cells are always present in the adult spleen, even in the absence of EMH, and the levels of *Scf* and *Cxcl12* expressed by individual stromal cells do not seem to increase following the induction of EMH[120]. However, endothelial cells and TCF21$^+$ perivascular stromal cells enter the cell cycle following the induction of EMH, and they expand in number as spleen cellularity increases[120]. Macrophages also promote the retention of HSCs and EMH in the spleen[128]. Thus, like the HSC niche in bone marrow, the EMH niche is complex and involves multiple cell types.

The expression of granulocyte colony-stimulating factor (G-CSF) is upregulated in monocytes and macrophages, as well as in various stromal cell populations in the bone marrow[129], as part of the response to sepsis and other inflammatory conditions[124]. The mechanism of G-CSF induction probably varies depending on the type of stress. However, during systemic bacterial infections, the ability to sense bacterial components (lipopolysaccharide and peptidoglycan) through Toll-like receptor activation is required for G-CSF induction and for the extramedullary accumulation of HSCs[124]. Increased G-CSF expression downregulates CXCL12 expression in the bone marrow, leading to HSC mobilization and bone marrow hypocellularity[124,130]. EMH typically involves myelopoiesis, erythropoiesis and thrombopoiesis, but the nature of the inducing stress determines which of these processes are involved. Stimulation with G-CSF in the setting of bacterial sepsis results mainly in myelopoiesis[124], whereas in pregnancy and severe anaemia, splenic EMH is shifted towards erythropoiesis[121].

Therapeutic implications

Insights gained from studying the HSC niche have already had an impact on autologous HSC transplants, which are an effective treatment strategy for patients with multiple myeloma, certain lymphomas and some diseases that can be treated with gene therapies. Owing to the potential for involvement of the bone marrow in these malignancies, HSCs are often mobilized to the peripheral blood, isolated and then re-infused into patients following ablative chemotherapy and/or radiation[131]. However, standard mobilization regimens involving G-CSF may not mobilize a sufficient number of CD34$^+$ HSCs for transplantation, particularly in older patients or in those who have undergone prior therapies. AMD3100, a small-molecule antagonist of CXCR4, has recently been approved by the US Food and Drug Administration (FDA) for use in this setting. Significantly more patients achieved adequate HSC mobilization when treated with AMD3100 and G-CSF than when treated with G-CSF alone[131]. Targeting additional niche factors may further increase the efficiency of HSC mobilization and provide a therapeutic benefit, particularly as transplanting higher doses of HSCs is associated with a faster recovery of blood cell counts, shorter periods of hospitalization and reduced transplant-related mortality[132].

The development and progression of primary bone marrow disorders — such as myeloproliferative neoplasms, myelodysplastic syndrome and acute myeloid leukaemia — are also affected by the interaction between malignant cells and the bone marrow microenvironment. Leukaemia cells remodel the HSC niche to promote tumour growth, as well as to inhibit residual haematopoiesis[133]. Leukaemia cells may promote EMH by altering the expression of niche factors; indeed, they have been shown to reduce the expression of CXCL12 in bone marrow stromal cells[134,135]. Increased bone marrow vascularization is common in these settings, and may correlate with more advanced disease, increased aggressiveness of the tumour cells and faster progression to fibrosis[136]. Altered sympathetic nerve signalling in the bone marrow, either as a result of the leukaemia itself or as a consequence of chemotherapy, seems to create a more favourable environment for leukaemia growth[106,135,137]. Engineered mutations in bone marrow stromal cells can non-cell-autonomously promote the generation of haematopoietic neoplasms in mice[138–141]. This is not likely to be a mechanism of leukaemogenesis in humans, in whom mutations would stochastically influence only small numbers of stromal cells. Nonetheless, it provides proof of principle that manipulation of the niche can have major non-cell-autonomous effects on neoplastic cells. Consistent with this, certain T cell acute lymphoblastic leukaemia cells cannot engraft after transplantation when *Cxcl12* is deleted from endothelial cells[142].

As supportive care has improved, bone marrow transplants and other forms of HSC transplantation have become safer and more widely used. For non-life-threatening conditions such as sickle cell anaemia, HSC transplants were rarely carried out 20 years ago, despite being potentially curative[143]. However, improvements in supportive care have reduced the risks of this procedure, and so transplants are increasingly being carried out using either allogeneic HSCs or gene-corrected autologous HSCs[144]. A better understanding of the HSC niche offers the opportunity to continue to improve supportive care and to identify

AMD3100
Also known as plerixafor. A CXC-chemokine receptor 4 (CXCR4) antagonist that is used to promote the mobilization of haematopoietic stem cells by reducing CXC-chemokine ligand 12 (CXCL12)–CXCR4 signalling.

Box 2 | **Important questions for future research**

- How does the niche change after myeloablation or irradiation? What mechanisms regulate the regeneration of haematopoiesis and haematopoietic stem cell (HSC) niches after injury? To address these questions, it will be necessary to systematically image the expression patterns of stem cell factor (SCF) and CXC-chemokine ligand 12 (CXCL12) in haematopoietic tissues after injury, and to conditionally delete these factors from candidate niche cells to functionally identify their key sources during regeneration. Once the relevant niche cells have been identified, it will be interesting to identify new mechanisms by which they promote haematopoietic regeneration.
- Are there undiscovered growth factors that regulate HSC maintenance or haematopoiesis? RNA sequencing of niche cells has the potential to identify such factors. Gene targeting approaches could then be used to test whether the new factors are required for specific aspects of HSC function or haematopoiesis.
- How much cross-regulation is there among cell types in the bone marrow? What is the proximity of niche cells to each other, and what might this mean for crosstalk with or co-regulation by various niche components? The conditional deletion of specific niche factors from specific cell types would make it possible to determine whether they have non-cell-autonomous effects on other cells in the bone marrow.
- Why are there 100 times fewer HSCs than stromal cells that express high levels of SCF and CXCL12 in the bone marrow? Is the HSC niche truly space-limited? Is it limited by competition from other primitive progenitors (that also depend on SCF and CXCL12), or is only a subset of niche cells competent to sustain HSCs at any one time?
- What are the mechanisms that regulate the activation of extramedullary haematopoiesis (EMH)? The identification of the cells that create the EMH niche in the spleen will make it possible to study new mechanisms by which they regulate EMH.
- Are there different perivascular domains that serve as niches for different types of haematopoietic progenitor? It will be important to identify additional markers that resolve functionally distinct subpopulations of mesenchymal stromal cells in the bone marrow.
- To what extent are HSCs and niche cells regulated by long-range signals that integrate HSC function with systemic physiological changes, such as pregnancy and nutrition?

new approaches that can promote haematopoietic regeneration after transplantation or chemotherapy, or in the context of benign haematopoietic diseases.

Conclusions

Advances in imaging and genetic tools have rapidly increased our understanding of HSC niches in the past 5 years, although there are many questions still to be answered (BOX 2). Dividing and non-dividing HSCs are maintained in perivascular niches. They are mainly associated with sinusoidal blood vessels in the adult bone marrow and spleen, but a subset of HSCs is most closely associated with arterioles. The periarteriolar and perisinusoidal microenvironments differ in terms of the capacity of HSCs to intravasate into the circulation and in terms of their exposure to blood plasma components. Endothelial cells and LEPR$^+$/CAR perivascular stromal cells are the main sources of factors (such as CXCL12 and SCF) that are known to be required for HSC maintenance in normal, young-adult bone marrow. However, several other bone marrow cell types — including megakaryocytes, monocytes and macrophages, neurons (specifically, nerve fibres) and Schwann cells —directly or indirectly regulate HSCs or niche function through other mechanisms. The vascular and stromal compositions of the bone marrow change during ageing. EMH in the spleen depends on a perivascular niche that is associated with sinusoids in the red pulp, in which endothelial cells and TCF21$^+$ stromal cells are the main sources of SCF and CXCL12. This niche is necessary for the recovery of haematopoiesis following stresses such as blood loss.

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