REVIEWS

Bone-marrow haematopoietic-stem-cell niches

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Abstract | Adult stem cells hold many promises for future clinical applications and regenerative medicine. The haematopoietic stem cell (HSC) is the best-characterized somatic stem cell so far, but *in vitro* expansion has been unsuccessful, limiting the future therapeutic potential of these cells. Here we review recent progress in characterizing the composition of the HSC bone-marrow microenvironment, known as the HSC niche. During homeostasis, HSCs, and therefore putative bone-marrow HSC niches, are located near bone surfaces or are associated with the sinusoidal endothelium. The molecular crosstalk between HSCs and the cellular constituents of these niches is thought to control the balance between HSC self-renewal and differentiation, indicating that future successful expansion of HSCs for therapeutic use will require three-dimensional reconstruction of a stem-cell–niche unit.

Self-renewal
The capacity of a stem cell to divide in such a way that one or both daughter cells retain the stem-cell fate.

Steel-Dickie mice
(*Sl/Sl^d*). A spontaneous mouse mutant with a defect in the production of membrane-bound stem-cell factor (SCF), although secreted SCF is produced normally

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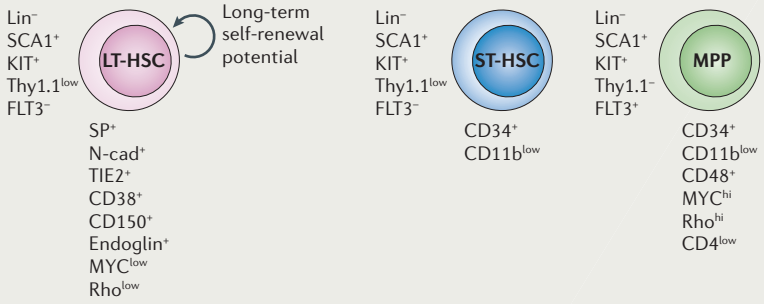
Adult stem cells are present in most self-renewing tissues, including the skin, the intestinal epithelium and the haematopoietic system. On a single-cell basis, they have the capacity both to produce more stem cells of the same type (that is, to self-renew) and to give rise to a defined set of mature differentiated progeny to maintain or repair their host tissue[1–3]. The best-characterized adult stem cell is the haematopoietic stem cell (HSC)[4,5]. Since HSCs were first identified[6], advances in technology have made it possible to purify adult mouse HSCs close to homogeneity. Several groups have achieved long-term reconstitution of the haematopoietic system of a lethally irradiated mouse by transplantation of a single purified bone-marrow HSC, providing functional proof of the existence of adult HSCs[2,7–9]. Maintenance of HSCs and regulation of their self-renewal and differentiation *in vivo* is thought to depend on their specific microenvironment, which has been historically called the haematopoietic-inductive microenvironment[10] or 'stem-cell niche'[11]. The crucial role of the microenvironment for HSC function has long been recognized because a mutation in the gene encoding membrane-bound stem-cell factor (SCF; also known as KIT ligand) that is present in *Sl/Sl^d* mice (steel-Dickie mice) causes changes in the HSC niche and leads to the failure of bone-marrow HSC maintenance *in vivo*[12–14]. Nevertheless, the structure and localization, as well as the molecular and cellular basis for niche activity, have long remained a 'black box'. It is only recently that the concept of a stem-cell niche has been supported by data on the molecules and cell types that are involved in its formation, first in invertebrates and more recently in mammals[1,15–17]. Many of the different types of signals that are exchanged between stem cells and niche cells, as well as some of the signalling pathways that control stem-cell maintenance, self-renewal and differentiation, have recently been identified. In this Review, we discuss models for the different types of bone-marrow HSC niches that might exist, particularly focusing on the molecules that are known to coordinate HSC function *in vivo*.

The adult HSC

Murine HSCs were initially identified on the basis of their ability to form colonies in the spleens of lethally irradiated mice following bone-marrow transfer[6,18]. Subsequently, a number of assays have been developed to monitor HSC activity *in vivo* and *in vitro* (BOX 1). The most widely accepted assay is the capacity of HSCs to provide lifelong reconstitution of all blood-cell lineages after transplantation into lethally irradiated recipients. The strictest version of this long-term repopulating (LTR) assay, known as serial transplantation, requires that HSC-containing donor bone marrow can be re-transplanted into secondary, and even tertiary, recipients while retaining both self-renewal and multi-lineage differentiation capacity[19]. These functional assays have been used to establish the cell-surface phenotype of mouse HSCs, allowing their prospective isolation by fluorescence-activated cell sorting (FACS) (BOX 1).

All functional HSCs are found in the population of bone-marrow cells that does not express the cell-surface

Box 1 | **Characteristics of haematopoietic stem cells**



Haematopoietic stem cells (HSCs) are defined functionally by their ability to mediate long-term repopulation of all blood-cell lineages (known as long-term repopulating (LTR) activity) and to form colony forming units in the spleen after transfer to lethally irradiated recipients. Assays to assess HSC activity *in vitro* include LTC-IC (long-term culture-initiating cell) and CAFC (cobblestone area-forming cell) assays[131].

All LTR HSCs are contained in the lineage-negative (Lin)⁻ stem-cell antigen 1 (SCA1)⁺KIT⁺ (LSK) subset that comprises ~0.5% of bone marrow[132]. 100 LSK cells are sufficient for multi-lineage LTR activity[23]. Additional markers to further subdivide the LSK population into long-term HSCs (LT-HSCs) and short-term HSCs (ST-HSCs), which have limited self-renewal activity, have been identified and are summarized in the figure. LTR activity is also enriched in the population of bone-marrow cells with low-level staining of rhodamine 123 (Rho)[133]. In addition, functional adult LTR HSCs can also be isolated by their ability to actively efflux the DNA-binding dye Hoechst 33342. This characteristic is designated as side-population (SP) ability[134,135].

Single-cell reconstitution studies have indicated the following frequencies for multi-lineage reconstitution and long-term engraftment:

- LSKThy1.1^{low} cells (18%)[2,7]
- SP⁺Rho^{low}Lin⁻ cells (40%)[136]
- LSKCD150⁺CD48⁻CD41⁻ cells (47%)[9]
- LSKSP⁺CD34⁻ cells (35%)[137] and (96%)[8]

LT-HSCs divide infrequently because (by DNA content) only ~5% are in the S or G_2/M phases of the cell cycle[51,138], and 60-70% of LSK cells are shown to be in G_0 by Ki67 staining[52]. Studies using bromodeoxyuridine (BrdU) uptake have calculated that LSK HSCs divide every 30–60 days[51,138]. 3.8% of LSK CD150⁺ HSCs are in the S or G_2/M phases of the cell cycle[9]. The low cycling status of HSCs might explain their significant resistance to cytotoxic drugs *in vivo*[40].

Label-retaining cells (LRCs) are defined by their capacity to retain the DNA label BrdU long-term (for 70 days). Lin⁻KIT⁺ LRCs are enriched for phenotypic HSCs, but due to the nature of the assay, functional LTR activity cannot be assessed.

LT-HSCs, ST-HSCs and haematopoietic progenitor cells show substantially different gene expression patterns[9,24–27].

FLT3, fms-related tyrosine kinase 3; MPP, multipotential progenitor; N-cad, N-cadherin; TIE2, tyrosine kinase receptor 2.

markers normally present on lineage (Lin)-committed haematopoietic cells but does express high levels of stem-cell antigen 1 (SCA1) and KIT. Therefore, this HSC-containing subset of bone-marrow cells is known as the LSK (Lin⁻SCA1⁺KIT⁺) subset. Because only some phenotypic LSK HSCs have LTR activity, they can be further subdivided into long-term (LT)-HSCs, which are CD34⁻ fms-related tyrosine kinase 3 (FLT3)⁻CD150⁺ and have LTR activity, and short-term (ST)-HSCs, which are CD34⁺FLT3⁻ and have only limited self-renewal activity[9,20–22] (BOX 1). Although it has been shown that 100 LSK HSCs can provide protection from lethal irradiation[23], several groups have succeeded in reconstituting all haematopoietic lineages from a single, purified HSC (BOX 1). These data clearly show that at the clonal-level HSCs fulfill the characteristics of true adult stem cells — multi-lineage reconstitution and long-term self-renewal. Recent gene-profiling studies have begun to establish a transcriptional signature of purified HSCs, which is the first step to elucidating the molecular mechanisms of HSC function[9,24–27]. Furthermore, the number of functional HSCs *in vivo* is altered in a large number of mutant mice (see Supplementary information S1 (table)), implicating several of these gene products in the regulation of self-renewal and differentiation of stem cells.

Asymmetric self-renewing division in stem cells

The vast majority of cell divisions are symmetrical, producing identical daughter cells and leading (in the absence of apoptosis) to increased numbers of cells. This process is readily observed for cells in culture and also occurs during organogenesis, where substantial cellular expansion (including stem cells) occurs during embryogenesis. By contrast, under homeostatic conditions in the adult, the number of tissue stem cells in a particular organ remains relatively constant, despite the fact that they proliferate, because they not only self-renew but also produce differentiated progeny.

This balance could be achieved if the number of stem cells dividing symmetrically to generate two identical daughter cells with stem-cell function was equivalent to the number of stem cells giving rise to two differentiated daughter cells. However, because this mechanism does not function at the single-cell level, and would require close coordination of two separate stem-cell populations, it is commonly assumed that an individual stem cell can give rise to two non-identical daughter cells, one maintaining stem-cell identity and the other becoming a differentiated cell. There are two mechanisms by which this asymmetry can be achieved, depending on whether it occurs pre- (divisional asymmetry), or post- (environmental asymmetry) cell division (FIG. 1).

Divisional asymmetry. In divisional asymmetry, specific cell-fate determinants in the cytoplasm (mRNA and/or proteins) redistribute unequally before the onset of cell division. During mitosis, the cleavage plane is oriented such that only one daughter cell receives the determinants. Therefore, two non-identical daughter cells are produced, one retaining the stem-cell fate while the other initiates differentiation (FIG. 1a).

In invertebrate model systems, the establishment of asymmetry by this mechanism is crucial for various developmental processes and the molecular basis for it has been well documented[28]. Asymmetrically localized proteins in *Drosophila melanogaster* include members of the partitioning defective (PAR) family of proteins, such as Inscuteable (INSC) and Partner of Inscuteable (PINS, the homologue of which is LGN in mammals), as well as NUMB, a negative modulator of Notch signalling[29]. However, only a few examples of divisional asymmetry have been documented in higher vertebrates[28,30]. For example, in the mammalian fetal epidermis, basal cells not only divide symmetrically to allow a two-dimensional expansion of the

epidermis, but also divide asymmetrically to promote stratification and differentiation of the skin. In this case, a protein complex that includes PAR3, LGN and a distant mouse homologue of *D. melanogaster* INSC (mINSC), forms an apical crescent that dictates the polarity of the ensuing cell division[30].

Although such a mechanism has not been shown in any vertebrate stem-cell type *in vivo*, a number of *in vitro* studies indicate that HSCs might undergo some type of asymmetric division. In an analysis of the ability of either of the two daughter cells derived from a single cultured HSC to long-term reconstitute lethally irradiated recipients, it was shown that ~20% of HSCs produced non-identical daughter cells[31–33]. However, these studies neither provide a mechanism for the observed asymmetry, nor show if it occurs pre- or post-cell-division. Moreover, whether these *in vitro* studies reflect the situation of bone-marrow HSCs remains unclear. Future studies will need to take advantage of recently developed tools to monitor asymmetric determinants such as mINSC and LGN[30] to determine whether, and to what extent, divisional asymmetry occurs in HSCs *in vitro*, and more importantly if it occurs in self-renewing HSCs in their niche.

Environmental asymmetry and the stem-cell niche concept. An alternative way to achieve asymmetry is by exposure of the two daughter stem cells to different extrinsic signals provided by distinct local microenvironments (FIG. 1b). Therefore, a stem cell would first undergo a symmetric self-renewing division, producing two identical daughter cells. While one daughter cell would remain in the niche microenvironment, conserving its stem-cell fate, the other would contact (passively or actively) a different microenvironment that would no longer preserve its stem-cell phenotype but would instead produce signals initiating differentiation[16,17]. Therefore, as with divisional asymmetry, the final product would be two non-identical daughter cells but achieved post-cell-division and not pre-cell-division (FIG. 1b).

Although the influence of the niche for stem-cell maintenance has been well documented, it has not been possible to monitor the division of vertebrate stem cells *in vivo*. However, recent studies of the mammalian epidermis indicate that the molecular mechanism for divisional asymmetry is conserved between invertebrates and vertebrates, raising the possibility that this mechanism might also mediate divisional asymmetry in mammalian stem cells (including HSCs). Therefore, it is possible that HSCs could undergo both divisional and environmental asymmetric divisions; therefore both mechanisms could be used in parallel by independent HSCs to direct non-stem-cell daughters to distinct cell fates.

Stem-cell-niche function

A stem-cell niche can be defined as a spatial structure in which stem cells are housed and maintained by allowing self-renewal in the absence of differentiation. Although the concept of the stem-cell niche was initially proposed in vertebrates[10,11], the *D. melanogaster* ovarian and testicular niches controlling germline stem-cell maintenance and differentiation were the first to be characterized[34,35].

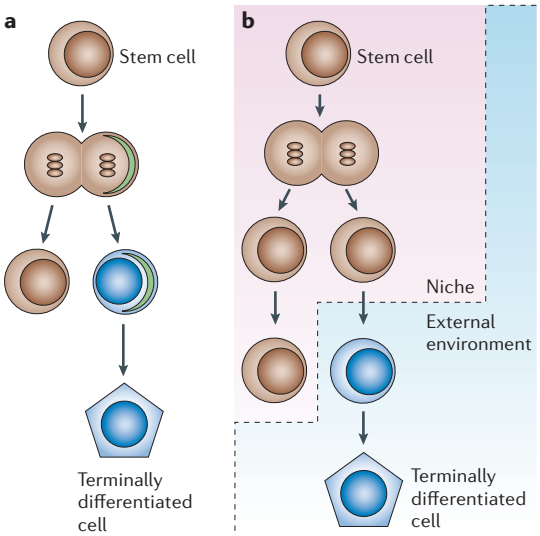


Figure 1 | **A model of asymmetric cell division.** **a** | During divisional asymmetry, cell-fate determinants are asymmetrically localized to only one of the two daughter cells, which retains stem-cell fate, while the second daughter cell differentiates. **b** | During environmental asymmetry, after division, one of two identical daughter cells remains in the self-renewing niche microenvironment while the other relocates outside the niche to a different, differentiation-promoting microenvironment.

In higher organisms, the analysis of stem-cell–niche interactions has been hampered by their unknown location. However, during the past few years, substantial progress has been made in localizing adult stem cells *in situ*. Many studies have indicated that most adult tissue stem cells (such as HSCs, or epidermal stem cells (ESCs) in the skin) divide infrequently and can be quiescent for weeks or even months[36–39]. In support of this notion, the adult-stem-cell pool is largely resistant to classical chemotherapeutic agents that target cycling cells[40]. In addition, HSCs that efficiently engraft after transplantation are mainly quiescent[41,42] and considered to be metabolically inactive[43]. Moreover, when the DNA of adult stem cells is labelled during cellular proliferation by nucleotide analogues (such as ^{3}H-thymidine or bromodeoxyuridine (BrdU labelling)), or by the histone H2B–enhanced-green-fluorescent-protein fusion protein (H2B–EGFP), the DNA label can be retained for months and has consequently been used to locate quiescent stem cells *in situ*[37–39,44,45]. For example, such label-retaining cells (LRCs) were initially identified using BrdU in the hair-follicle bulge in the skin, leading to the suggestion that ESCs were present in this structure[37,46]. However, the nature of this assay precludes a functional assessment of stem-cell activity post-identification, because to identify BrdU^{+} cells, the cells must be fixed. Subsequently, H2B–EGFP was used to show that LRCs in the bulge are indeed functional ESCs[38].

In the bone marrow, only BrdU^{+} LRCs have been identified in trabecular bone[39,45]. Nevertheless, in analogy to ESCs, BrdU^{+} LRCs in the bone marrow are probably highly enriched for functional HSCs, particularly if they also fail to express differentiation markers, although this

BrdU labelling
Incorporation of bromodeoxyuridine (BrdU) into newly synthesized DNA permits indirect detection of proliferating cells using fluorescently labelled BrdU-specific antibodies by either flow cytometry or fluorescence microscopy.

Trabecular bone
Also known as cancellous bone, this is found in areas of rapid turnover such as the ends of the long bones.

remains to be shown definitively. If so, these long-term quiescent HSCs are unlikely to contribute substantially to the normal homeostasis of the haematopoietic system with its high turnover rate. Instead, they might serve as a reserve pool that can be reactivated in response to stress or injury and might even be stored in a separate 'quiescent-storage' niche[16] (FIG. 2a). In response to myeloablative agents, HSCs are released into the circulation (a process known as mobilization, as discussed later), enter the cell cycle to re-establish haematopoiesis and migrate to putative HSC niches in the spleen and liver. After repair, they return to their bone-marrow niches and become quiescent again[27,47,48].

Although most adult stem cells are considered to be quiescent, this is not a requirement for all stem cells. For example, embryonic stem cells have enormous proliferative potential but retain their stem-cell fate, and fetal-liver HSCs, although highly proliferative, very efficiently reconstitute irradiated adult hosts[49,50]. Therefore, cell-cycle status might only reflect differences between fetal (that is, clonally expanding) HSCs and adult (that is, steady-state) HSCs rather than be a measure of stem-cell fate. Moreover, even during homeostasis, a proportion of stem cells are expected to divide at least occasionally (particularly in highly regenerative tissues such as the haematopoietic system), to maintain a constant flow of short-lived progenitors that can generate enough cells to replace those that are constantly lost during normal turnover. Indeed, continuous BrdU labelling has revealed a considerable number of cycling HSCs[51,52].

It is currently unclear whether all postulated stem-cell-niche functions (storage of quiescent stem cells, self-renewal and inhibition of differentiation) can be provided by a single niche, or whether different types of niches coexist. The main function of a self-renewing niche (FIG. 2b) would be to guarantee that (by environmental and/or divisional asymmetry) one of the two daughters of a dividing stem cell maintains the stem-cell fate while the other produces differentiating progenitors[53]. Such a self-renewing stem-cell niche would be more complex than a quiescent-storage niche but would be the essential unit that maintains normal tissue homeostasis. In this type of niche, one can propose that quiescent stem cells would be anchored in the centre of the niche, whereas self-renewing stem cells would be located close to the border separating the niche from the non-niche microenvironment, which could provide signals that would induce differentiation and/or cell division (FIG. 2b).

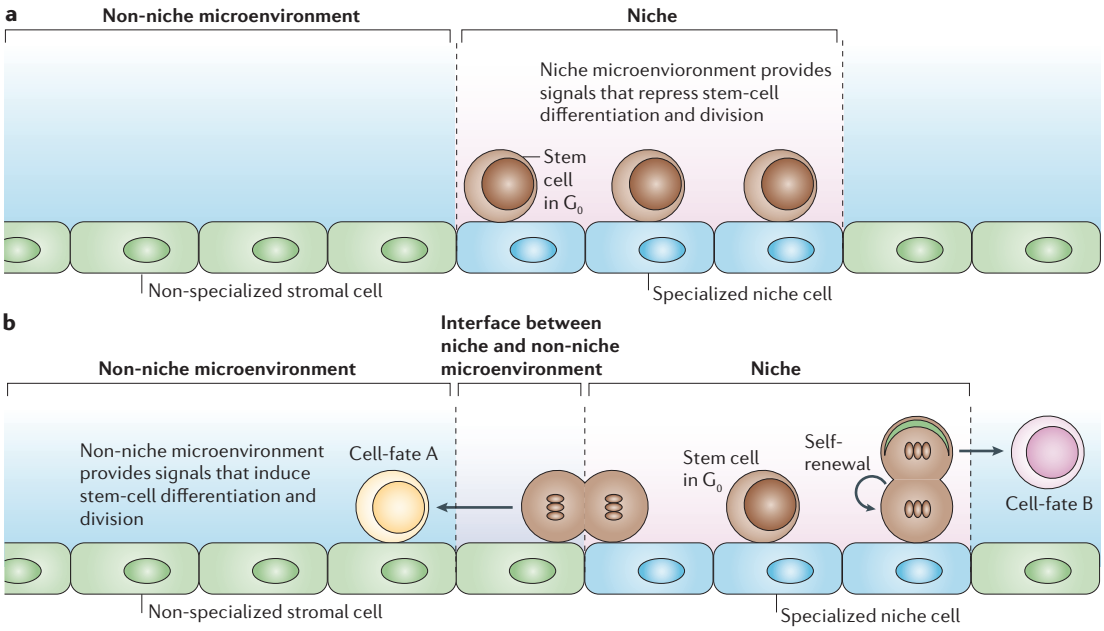


Figure 2 | **Different types of niche. a** | Quiescent-storage niche. Resting (G_0) stem cells are stored in 'quiescent' niches. Specialized niche cells generate a differentiation- and/or division-repressive environment. Under conditions of stress these might be mobilized to generate mature cells as required, and might then return to empty niches for storage or self-renewal. **b** | Self-renewing niche. Quiescent stem cells would be anchored in the centre of the niche, whereas self-renewing stem cells would be located close to the border separating the niche from the non-niche microenvironment (NNM). At this interface, niche signals (differentiation and/or division repression) and NNM signals (differentiation and/or division induction) intermingle to form a signalling centre. The appearance of a stem cell at the niche edge would expose it to proliferative and anti-adhesive signals emanating from the NNM. At the onset of cell division, one daughter cell would transit the interface towards the NNM to initiate differentiation, while the other would remain in the niche as a self-renewing stem cell, thereby achieving asymmetric division by environmental asymmetry[53]. Alternatively, signals from the periphery might induce asymmetric division of a formerly quiescent stem cell by polarization of determinants (right)[30]. Attachment of stem cells to niche cells (and possibly signals exchanged between them) would maintain stem-cell fate, while the budding daughter cell would initiate differentiation. Both mechanisms of asymmetrical division might occur in parallel, therefore allowing initiation of differentiation of stem cells to distinct cell fates (A and B).

Myeloablative agents
Used to completely or partially eliminate the haematopoietic system. These agents include the use of whole-body irradiation or cytotoxic drugs such as 5-fluorouracil.

Table 1 | **Mouse models affecting bone development and haematopoiesis**

Model	Further details of model	*In vivo* effects	Refs
Decreased number of HSCs			
Sl/Sl^{d} (steel-Dickie) mice	Spontaneous mutant mouse strain lacking membrane-bound SCF but not soluble SCF	Decreased osteoblast development, compromised haematopoiesis and HSC self-renewal	13,14,117
CBFα1-deficient mice	Knockout mice	No osteoblasts, no bone or bone-marrow development and no adult haematopoiesis	56,139,140
Col1α1–thymidine-kinase transgenic mice	Osteoblast-specific expression of thymidine kinase allows conditional ablation of osteoblasts following administration of ganciclovir (Cytovene, Roche)	Reversible depletion of osteoblasts and haematopoietic progenitors or HSCs	69,70
Increased number of HSCs			
Intravenous administration of PTH	N/A	Increased number of osteoblasts and HSCs but not other haematopoietic-cell lineages	67
Conditional BMPR1A-deficient mice	Inducible deletion of *Bmpr1a* in bone-marrow stroma	Increased number of osteoblasts and HSCs	39
Col1α1–constitutively-active-PPR transgenic mice	Osteoblast-specific expression of constitutively active PPR	Increased number of osteoblasts and HSCs	67

BMPR1A, bone morphogenetic protein receptor 1A; CBFα1, core binding factor α1; Col1α1, type 1 collagen α1; HSC, haematopoietic stem cell; N/A, not applicable; PRR, PTH/PTH-related protein receptor; PTH, parathyroid hormone; SCF, stem-cell factor.

The bone-marrow HSC niche

The link between bone-marrow formation (haematopoiesis) and bone development (osteogenesis) was first recognized in the 1970s in studies showing that first bone and then vascularized bone marrow developed after subcutaneous transfer of total, unmanipulated bone marrow[54,55]. The term 'niche' for the specific HSC bone-marrow microenvironment was first coined by Schofield, who proposed that HSCs are in intimate contact with bone, and that cell–cell contact was responsible for the apparently unlimited proliferative capacity and inhibition of maturation of HSCs[11].

More recently, several mutant mice in which haematopoiesis is defective as a consequence of primary defects in bone development or remodelling, have implicated osteoblasts and/or osteoclasts in the formation and function of the bone-marrow HSC environment or niche (TABLE 1). For example, mice lacking core binding factor α1 (CBFα1; also known as RUNX2), which is one of the earliest osteoblast-specific transcription factors, have defective bone-marrow haematopoiesis and extensive extramedullary haematopoiesis, owing to defects in osteoblast differentiation and the consequent failure to form bone[56,57] (see Supplementary information S1 (table)). However, whether the haematopoietic deficiency is a secondary effect caused by the absence of a suitable bone-marrow cavity or whether the deficiency in CBFα1 directly affects haematopoiesis remains unclear. In this respect, several other mouse mutants with defects in bone development and/or remodelling have been described[58] but potential effects on haematopoiesis have not been documented.

The physical location of HSCs close to the bone surface was first shown in 1975 (REF. 59). Morphological evidence for the presence of HSC niches in close association with the endosteum was provided more recently when HSC or haematopoietic progenitor activity and/or phenotype (TABLE 2) were shown to localize close to the endosteal lining of bone-marrow cavities in trabecular regions of long bones, whereas more differentiated haematopoietic progenitors were found mainly in the central bone-marrow region[9,39,52,59–63]. For example, 89% of CD45^{+}Lin^{-} LRCs were shown to be attached to the endosteal surface, and only 11% of these cells were in the centre of the bone marrow[39]. However, in another study, although 57% of a bone-marrow population highly enriched in LTR activity and defined as CD150^{+}CD48^{-}CD41^{-}Lin^{-} (denoted CD150^{+} HSCs) were located in trabecular bone, only 14% were found at the endosteum and the rest were found at bone-marrow sinusoids (as discussed later)[9].

The discrepancy between these two studies[9,39] might be a reflection of the different criteria used to identify HSC subsets *in situ*, which could mean that the populations contain different proportions of functional, or quiescent versus self-renewing, HSCs (TABLE 2). Nevertheless, HSCs are likely to be located in close proximity to bone surfaces, supporting the concept of an endosteal niche.

The endosteal bone-marrow HSC niche. The first direct evidence for cells involved in bone formation having stem-cell-supporting activity was provided by studies in which both mouse and human osteoblast cell lines were shown to secrete a large number of cytokines that promote the proliferation of haematopoietic cells in culture[64]. Furthermore, long-term bone-marrow cultures contain osteoblasts, and many, but not all, stromal cell lines that have been shown to maintain HSCs *in vitro* also show bone-formation activity[64–66].

A direct role for the involvement of osteoblasts in HSC regulation and/or maintenance *in vivo* has recently been obtained from two studies in which osteoblast numbers were experimentally increased or decreased. In the first study[67], osteoblast-specific expression of a constitutively active form of parathyroid hormone (PTH) or the PTH/PTH-related protein receptor (PPR), which is an important regulator of calcium homeostasis, and therefore bone formation and resorption, was achieved using the type 1 collagen α1 (*Col1a1*) promoter. This

Osteoblasts
Mesenchymal cells that produce bone matrix that forms bone after mineralization.

Osteoclasts
Large, multi-nucleated cells derived from macrophages that resorb bone. The activity of osteoblasts and osteoclasts form an equilibrium that maintains bone during homeostasis and remodelling.

Endosteum
The cellular lining separating bone from bone marrow. It comprises different cell types including osteoblasts, osteoclasts and stromal fibroblasts.

Bone-marrow sinusoids
Low-pressure vascular channels surrounded by a single layer of fenestrated endothelium.

Table 2 | **Localization of HSCs or haematopoietic progenitor cells in the bone marrow**

Cell population	Source*	Assay	Result	Refs
Femoral bone marrow	Endogenous	CFUs	• 3-fold increase in CFU activity in endosteal bone marrow compared with central bone marrow	59
Endosteal bone-marrow cells identified as Lin^-Sudan-Black-staining, and with lymphoid morphology	Endogenous	Scanning electron microscopy and histology of opened rat bone. CFUs of endosteal bone marrow	• Lin^- Sudan-Black-staining cells morphologically resembling lymphocytes located close to endosteum • High frequency of CFUs from endosteal bone marrow	60
Lin^- bone marrow	Transplanted	*In situ* localization of CFSE-labelled cells transferred into sub-lethally irradiated hosts	• 15h post-transplantation CFSE-labelled cells attached to $BMPR1A^+OPN^+N\text{-}cad^+$ osteoblasts at endosteum	52
Lin^-Rho^{low} $WGA^{low/int}$ bone marrow	Transplanted	*In situ* localization of CFSE-labelled cells 10–15h post transfer into non-ablated recipients	• >60% CFSE-labelled cells at endosteum post-transplantation	63
LSK HSCs mixed with Lin^-SCA1^+ and Lin^-KIT^+ (HSPCs)	Transplanted	Intravital bone-marrow imaging (calvarial bone) of transferred DiR-labelled cells	• Attachment of HSPCs to $CXCL12^+$ vascular microdomains in the perivascular space 2h after transfer • No quantitation	62
Lin^-CD45^+ LRCs ($BrdU^+$). At least 50% are also $SCA1^+KIT^+$	Endogenous	*In situ* localization by immuno-histochemistry	• 6-fold increase of $BrdU^+Lin^-CD45^+$ LRCs at endosteum compared with bone marrow centre • LRCs attached to $N\text{-}cad^+$ osteoblasts	39
$Lin^-SCA1^+CD41^-CD45^+CD48^-$ $CD150^+$ bone marrow or mobilized spleen	Endogenous	*In situ* localization by immuno-histochemistry	• 57% in trabecular bone, 14% at endosteum • 60% associated with $MECA\text{-}32^+$ sinusoidal epithelium in bone marrow • 100% in contact with or close to $MECA\text{-}32^+$ sinusoidal epithelium in mobilized spleen	9

**In situ* detection of endogenous or transplanted HSCs or haematopoietic progenitor cells. BMPR1A, bone morphogenetic protein receptor 1A; BrdU, bromodeoxyuridine; CFSE, 5(6)-carboxyfluorescein diacetate succinimidyl ester; CFUs, colony-forming units in the spleen; CXCL12, CXC-chemokine ligand 12; DiR, dialkylcarbocyanine; HSPCs, haematopoietic stem and progenitor populations; Lin^-, lineage negative; LRCs, label-retaining cells; LSK, $Lin^-SCA1^+KIT^+$ cells; MECA-32, a pan-endothelial marker; N-cad, N-cadherin; OPN, osteopontin; Rho, rhodamine 123; SCA1, stem-cell antigen 1; WGA, wheat germ agglutinin.

resulted in a simultaneous increase in the number of both osteoblasts and HSCs in the bone marrow. Moreover, the maintenance of HSCs *in vitro* was more efficient when supported by stromal cells that were isolated from these transgenic mice, presumably because of an increase in the proportion of osteoblasts in the stromal-cell population compared to stromal cells from wild-type mice[67].

In a second study[39], mice lacking bone morphogenetic protein (BMP)[68] receptor 1A (BMPR1A, which is normally expressed on osteoblasts lining the endosteum) in the bone-marrow stroma showed a simultaneous increase in the number of both osteoblasts and repopulating HSCs, although the number of more differentiated cells remained unchanged[39]. Moreover, Lin^- LRCs and osteoblasts were shown to be in direct contact through homotypic N-cadherin interactions (TABLE 2). Therefore, specialized spindle-shaped N-cadherin-expressing osteoblasts (SNOs) located in the endosteum were postulated to be essential components of the HSC bone-marrow niche (FIG. 3). Both studies[39,67] show that an increase in the number of osteoblasts directly correlates with the number of functional LTR HSCs, indicating that osteoblasts (or a subset of these cells) are an essential part of the niche and are limiting for niche size and/or activity. This concept is supported by experiments in which osteoblasts were conditionally ablated by targeting the expression of thymidine kinase (which induces cell death in response to ganciclovir (Cytovene, Roche), to the osteoblast lineage[69,70]. In these mice, progressive bone loss is accompanied by a decrease in bone-marrow cellularity, including a decrease in the number of LSK HSCs. Importantly, in response to the loss of osteoblasts, progenitor cells (and presumably HSCs) are now found in the liver, spleen and peripheral blood. This type of extramedullary haematopoiesis is a typical response to bone-marrow stress. Interestingly, osteoblast depletion due to thymidine-kinase activity is reversible following the removal of ganciclovir and is accompanied by a corresponding re-emergence of bone-marrow haematopoiesis. By contrast, genetic ablation of osteoblasts using the osteocalcin promoter (which is active at a later stage of osteoblastogenesis than the *Col1a1* promoter) to drive thymidine-kinase expression has no effect on haematopoiesis[71], indicating that niches comprise immature osteoblasts. Although N-cadherin-expressing $PPR^+BMPR1A^+$ osteoblasts seem to be necessary and rate-limiting for niche function, it is probable that other cell types, such as osteoclasts, stromal fibroblasts and endothelial cells, also contribute to niche formation, activity or architecture.

The vascular bone-marrow HSC niche. The presence of a second specialized HSC microenvironment in the bone marrow has recently been postulated, as a large proportion of $CD150^+$ HSCs were observed to be attached to the fenestrated endothelium of bone-marrow sinusoids[9] (TABLE 2). A close interaction between HSCs and endothelial cells is not unexpected because both lineages arise from a common embryonic precursor, the haemangioblast[72]. Moreover, cell lines or purified primary endothelial cells that are derived from the yolk sac or the aorta–gonad–mesonephros promote the maintenance, or even clonal expansion,

Bone morphogenetic protein
Induces the formation of bone and cartilage, and is a member of the transforming growth factor-β (TGFβ) superfamily.

Stromal fibroblasts
Part of the endosteal lining separating bone and bone marrow.

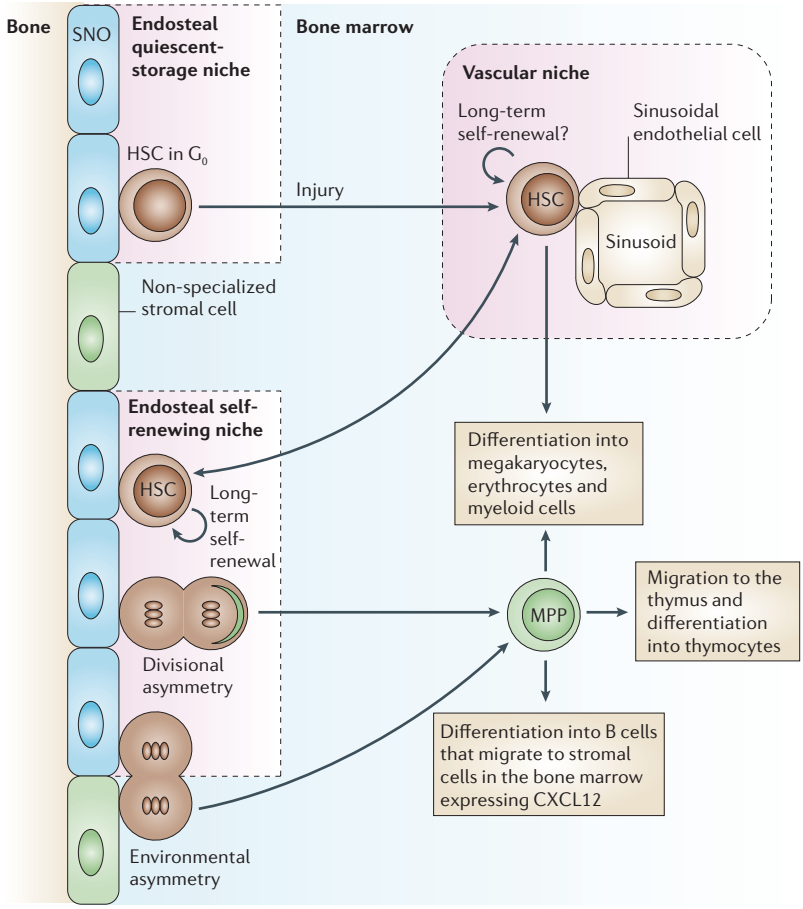


Figure 3 | **Model of bone-marrow HSC niches.** Endosteal bone surfaces are lined with stromal cells. Spindle-shaped N-cadherin-expressing osteoblasts (SNOs) serve as niche cells to maintain quiescence and prevent differentiation of attached haematopoietic stem cells (HSCs). The quiescent endosteal niche would maintain dormant HSCs long-term. In response to injury, quiescent HSCs might be activated and recruited to the vascular niche. The self-renewing niche would contain quiescent HSCs intermingled with dividing HSCs. Self-renewing HSCs produce multipotential progenitors (MPPs) either by divisional or environmental asymmetry. More HSCs can be generated by symmetrical divisions which might provide the vascular niche with new HSCs. Whether HSCs long-term self-renew in the vascular niche remains to be determined, and it is probable that influx of HSCs from endosteal niches is necessary to ensure prolonged haematopoietic-cell production at the vascular niche. HSCs in the vascular niche promote differentiation and expansion along megakaryocytic and other myeloid-cell lineages, particularly in response to injury. MPPs can give rise to all haematopoietic lineages, including B-cell precursors attached to randomly distributed CXC-chemokine ligand 12 (CXCL12)-expressing stromal cells that constitute a B-cell niche[82]. Unidentified T-cell precursors migrate to the thymus where they enter a microenvironment, promoting T-cell maturation.

of adult LSK HSCs *in vitro*[73,74]. By contrast, vascular endothelial cells that are isolated from various adult non-haematopoietic organs have little or no ability to maintain HSCs *in vitro*[75]. Therefore, bone-marrow sinusoidal endothelial cells (BMECs) are functionally and phenotypically distinct from microvasculature-endothelial cells of other organs[76]. Indeed BMECs constitutively express cytokines such as CXC-chemokine ligand 12 (CXCL12) and adhesion molecules such as endothelial-cell (E)-selectin and vascular cell-adhesion molecule 1 (VCAM1) that are important for HSC mobilization, homing and engraftment[62,77,78] (FIG. 4).

Mobilization
The efflux of haematopoietic stem cells from the bone marrow into the vasculature in response to bone-marrow stress or injury, or after treatment with cytokines such as granulocyte colony-stimulating factor (G-CSF).

A vascular bone-marrow HSC niche has previously been predicted to form during HSC mobilization after myeloablation. Quiescent HSCs detach from the endosteal niche and migrate towards the centre of the bone marrow to the vascular zone from where they re-establish haematopoiesis[76,77,79]. The recent finding that $CD150^+$ HSCs are attached to the sinusoidal endothelium now raises the possibility that a vascular bone-marrow HSC niche might also exist during homeostasis[9]. Why have two apparently distinct HSC niches in the bone marrow? Putative HSCs that have been identified by LRC assays are almost exclusively located in the endosteal niche[39], indicating that this niche might contain the most dormant HSCs and therefore serve as a quiescent-storage niche, or a self-renewing niche comprising both quiescent and self-renewing HSCs. In contrast to label-retaining HSCs that have not divided for many weeks, the $CD150^+$ HSC population comprises both long-term quiescent and self-renewing HSCs, because 3.8% of the cells are proliferating at any given time[9]. Because many of the proliferating cells are in contact with BMECs, it is probable that the vascular bone-marrow HSC niche contains self-renewing, rather than long-term dormant, HSCs. The location of $CD150^+$ HSCs — in close proximity to sinusoids — would enable them to constantly monitor the concentration of blood-borne factors that reflect the status of the haematopoietic system. Under haematological stress, a rapid and robust response could be mounted, and if necessary more HSCs could be recruited from endosteal niches (FIG. 3).

BMECs are known to support the survival, proliferation and differentiation of myeloid and megakaryocyte progenitors[76,77,80], whereas primary bone-marrow stromal cells release factors that inhibit megakaryocyte maturation[81]. These data indicate that megakaryocyte lineage development (and possibly the development of other myeloid-cell types) might be predominantly initiated at the vascular niche[76] (FIG. 3). It is probable that the pool of HSCs located in the vascular and self-renewing endosteal niches are freely exchanged to maintain homeostasis in a constantly changing haematopoietic environment. In addition, HSCs that are located in the self-renewing endosteal niche produce multipotential progenitors (MPPs) by divisional and/or environmental asymmetry (FIG. 3). These cells give rise to myeloid-cell lineages as well as lymphocyte precursors. B-cell progenitors are uniformly distributed throughout the bone marrow attached to CXCL12-expressing fibroblasts (in the B-cell niche)[82,83]. Because deletion of osteoblasts results in extramedullary haematopoiesis[70], the vascular bone-marrow HSC niche alone might not be sufficient to maintain long-term haematopoiesis. This indicates that in the bone marrow the vascular niche might be a secondary niche, requiring an influx of HSCs from the primary endosteal niches (FIG. 3). Collectively, the vascular and endosteal niches strongly cooperate to control HSC quiescence and self-renewing activity (and therefore HSC number), as well as the production of early progenitors to maintain homeostasis or re-establish it after injury.

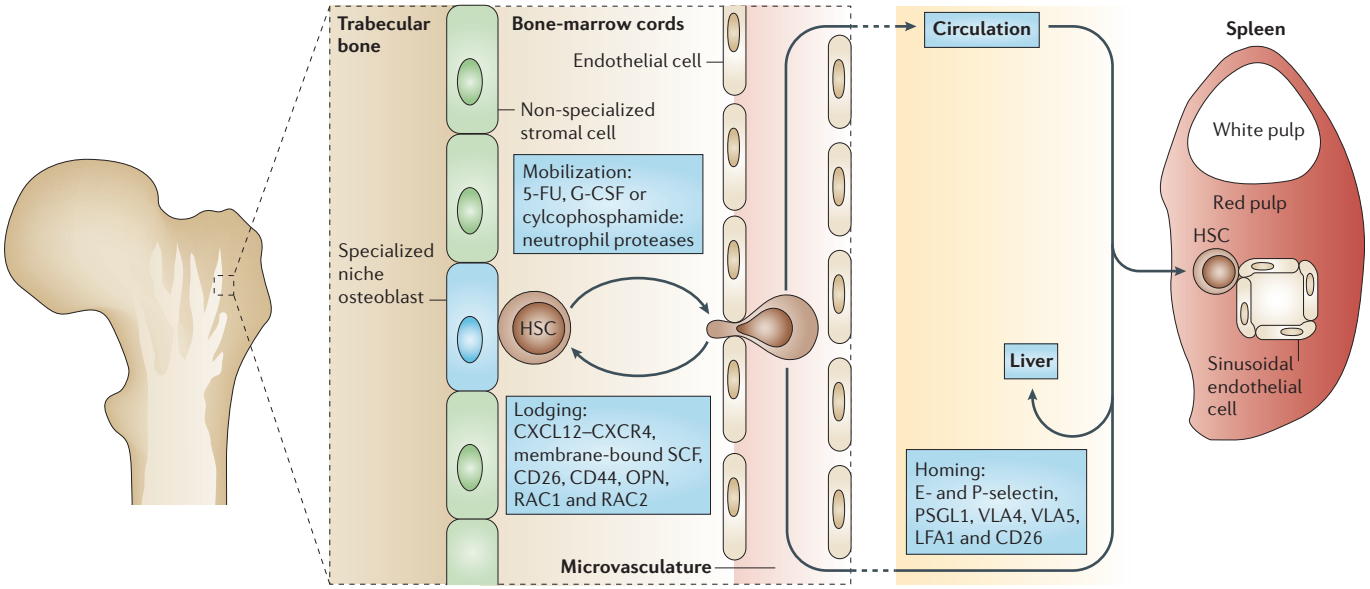


Figure 4 | **Mobilization, homing and lodging.** Schematic diagram showing some of the factors implicated in each process. Haematopoietic stem cells (HSCs) bound to the bone-marrow niche are mobilized in response to granulocyte colony-stimulating factor (G-CSF) or cyclophosphamide, or after peripheral myeloablation following treatment with 5-fluorouracil (5-FU). After extravasation from the bone-marrow cords into the microvasculature, HSCs enter the circulation and are distributed to peripheral tissues such as the spleen or liver. HSCs locate close to endothelial cells in the splenic red pulp. They home to the bone-marrow cords through the circulation, a process that is controlled by a number of adhesion molecules such as very late antigen 4 (VLA4), VLA5, lymphocyte function-associated antigen 1 (LFA1) or selectins. After entering the bone marrow, HSCs specifically lodge in the niche, a process requiring membrane-bound stem-cell factor (SCF), CXC-chemokine ligand 12 (CXCL12), osteopontin (OPN), hyaluronic acid, and their corresponding receptors. CXCR4, CXC-chemokine receptor 4; E-selectin, endothelial-cell selectin; P-selectin, platelet selectin; PSGL1, P-selectin glycoprotein ligand 1.

Entering and exiting the HSC niche

Although the vast majority of HSCs in the adult mouse are located in the bone marrow, HSCs show remarkable motility. In response to specific signals they can exit and re-enter the endosteal bone-marrow HSC niche, processes known as mobilization and homing, respectively (FIG. 4). These opposing biological processes are controlled by overlapping but distinct molecular mechanisms[84–86]. Massive mobilization of HSCs occurs in response to treatment with cyclophosphamide and granulocyte colony-stimulating factor (G-CSF), or bone-marrow injury. This is mediated by the release of neutrophil proteases, which lead to the degradation of niche-retention signals and adhesive connections (such as those provided by membrane-bound SCF, VCAM1 and CXCL12)[87] (FIG. 4). During extramedullary haematopoiesis (which can occur in the liver and spleen), extramedullary long-term self-renewal of HSCs might occur, so HSC niches should also be present in the spleen. Support for this idea comes from immunohistochemical analysis showing that two out of three mobilized CD150$^+$ HSCs in the spleen are in contact with sinusoidal endothelial cells[9]. Whether mobilized HSCs are retained in these locations in situations of sustained extramedullary haematopoiesis, and whether these niches are functionally equivalent to those present in the bone marrow, remains to be shown.

The release of HSCs not only occurs during mobilization but is also observed during homeostasis, when a small number of HSCs are constantly released into the circulation[88]. Although their precise physiological role remains unclear, they might provide a rapidly accessible source of HSCs to repopulate areas of injured bone marrow[87]. Alternatively, circulating HSCs might be a secondary consequence of permanent bone remodelling that causes constant destruction and formation of HSC niches, therefore requiring frequent re-localization of HSCs.

Transplanted HSCs have the capacity to home back to and lodge in bone-marrow niches. Homing can be defined as recruitment of circulating HSCs to the bone-marrow microvasculature and subsequent transendothelial migration into the extravascular haematopoietic cords of the bone marrow[15] (FIG. 4). Several cell-surface adhesion molecules, including selectins and integrins, are crucial for homing of HSCs to the bone-marrow HSC niche[84,85]. For example, β_1-integrin-deficient HSCs fail to migrate to the bone marrow after transfer[89]. Although homing is thought to be an unselective process that occurs at a similar frequency for most haematopoietic cell types, transendothelial migration into the extravascular haematopoietic cords of the bone marrow and subsequent lodging in endosteal bone-marrow HSC niches is a specific property of HSCs[15,90,91]. Subsequent engraftment is accompanied by the generation of a large number of haematopoietic progenitors and differentiated cells.

One crucial factor involved in migration, retention and mobilization of HSCs during homeostasis and after injury is CXCL12 (FIG. 4), which is expressed by several types of bone-marrow stromal cell, including osteoblasts

Homing
The specific movement or migration of haematopoietic stem cells through the vasculature to the bone marrow.

Engraftment
The production of more haematopoietic stem cells by symmetrical divisions and production of a large number of progenitors and differentiated cell types.

LRC assay
(Label retaining cell assay). Identifies long-lived quiescent cells such as adult stem cells. They can be visualized *in situ* by pulse labelling of their DNA with BrdU (or ^{3}H-thymidine or a histone H2B–EGFP transgene) followed by a chase period of a month or more. Detection of BrdU$^+$ cells requires fixation, precluding subsequent functional analysis.

and vascular endothelial cells[92,93]. Similar to SCF, CXCL12 expression and secretion is induced in response to haematopoietic-cell loss due to irradiation, chemotherapy or hypoxia, and purified HSCs migrate specifically towards CXCL12 but not towards any other single chemokine[94]. The biological effects of CXCL12 are mediated by its capacity to induce motility, chemotaxis and adhesion, as well as to induce secretion of matrix metalloproteinases (MMPs) and angiogenic factors (such as vascular endothelial growth factor (VEGF)) by cells expressing its receptor, CXC-chemokine receptor 4 (CXCR4). Mice lacking either CXCL12 or CXCR4 show similar embryonic lethal defects, including impaired myeloid- and B-cell haematopoiesis[87,95,96] (see Supplementary information S1 (table)). Importantly, CXCL12 is not essential for HSC generation or expansion in the fetal liver but is crucial for the colonization of bone marrow during late fetal development. Collectively, the genetic and functional data indicate that the CXCL12–CXCR4 pathway is crucial for retention and maintenance of adult HSCs.

The cytoskeleton also cooperates with cell-surface adhesion molecules to regulate migration and adhesion, and is essential for homing and mobilization. For example, Lin^-KIT^+ cells lacking the RHO family GTPase RAC1 not only fail to engraft[97], but also have reduced homing efficiency to the bone marrow and endosteum. Moreover, the deletion of both RAC1 and RAC2 causes massive defects in HSC or haematopoietic-progenitor-cell proliferation, survival, adhesion to very late antigen 4 (VLA4) and/or VLA5, and migration towards CXCL12 *in vitro*. Deletion of both RAC1 and RAC2 in engrafted HSCs *in vivo* leads to a massive mobilization of HSC or haematopoietic progenitor cells to the peripheral blood. Together, these data indicate that RAC1 and RAC2 have essential roles in homing, lodging and retention of HSCs in the endosteal bone-marrow HSC niche[86,97] (see Supplementary information S1 (table)). In summary, a complex combination of migration, adhesion, proteolysis and signalling occurs at the interface between HSCs and the endosteal bone-marrow niche (FIGS 4,5), and signals originating from the periphery can influence HSC homing, retention and mobilization, therefore determining whether a niche is silent or whether HSCs exit the niche in response to stress.

Molecular crosstalk in the endosteal niche

Although little is known about the signals that are exchanged between HSCs and osteoblasts *in situ*, several receptors, membrane-anchored proteins and secreted factors are expressed by both cell types[65]. Comparative gene-expression profiling has recently been performed on HSC-supporting and non-supporting stromal cell lines, identifying a number of new molecules that might regulate endosteal bone-marrow HSC-niche activity. These include various interleukins, oncostatin-M, ciliary neurotrophic factor and the membrane protein mKirre[98]. Here, however, we will focus on the role of molecules for which genetic or functional evidence has been shown *in vivo* for the regulation of HSC function and/or niche activity (FIG. 5) (see Supplementary information S1 (table)).

Notch signalling. Signalling through Notch receptors is involved in many cell-fate decisions and is thought to have a role in the maintenance of stem cells in a variety of tissues[99,100]. Moreover, several Notch receptors and Notch-receptor ligands are expressed in the bone marrow[101], leading to the suggestion that Notch signalling has a role in HSC self-renewal and/or clonal expansion. Support for this hypothesis has been provided by *in vitro* culture of purified HSCs on various stromal cell lines[102,103]. In addition, overexpression of Notch1 in recombination-activating gene 1 (RAG1)-deficient Lin^-SCA1^+ progenitors resulted in an increase in the number of HSCs or haematopoietic progenitor cells *in vitro* and *in vivo*[104]. Moreover, as expression of the Notch ligand Jagged-1 is upregulated on osteoblasts that are exposed to PTH, the concomitant increase in HSCs has been postulated to be caused by increased Notch signalling[67]. However, in contrast to studies leading to over-activation of Notch signalling, loss-of-function studies have failed to show any requirement for Notch signalling in HSCs. Conditional knockout mice for Jagged-1, Notch-1 and Notch-2, or CSL (the common mediator of all signalling through Notch receptors) have all been shown to be dispensable for HSC and niche function *in vivo*[105–108]. Together, these data indicate that signalling events occurring between HSCs and osteoblasts are more complex than has been previously assumed and involve factors other than Notch signals.

Osteopontin. One mechanism by which osteoblasts might regulate the number of HSCs in the bone marrow is through secretion of osteopontin (OPN), an acidic glycoprotein, into the bone matrix[109]. OPN-deficient mice have a two-fold increase in HSCs and, because the same effect was observed by transplanting wild-type HSCs into lethally irradiated OPN mutant recipients, OPN production by osteoblasts has a negative effect on HSC number[91,110]. Because cultured Lin^-SCA1^+ bone-marrow cells are induced to undergo apoptosis when exposed to soluble OPN, the increase in the number of HSCs in OPN-deficient mice has been postulated to be a result of decreased apoptosis[110]. In addition, OPN has been postulated to act as a negative regulator of HSCs by actively maintaining their quiescence[91].

Membrane-bound SCF. The steel (*Sl*) locus encodes both membrane-bound SCF and secreted SCF. The latter is produced by alternative splicing followed by proteolytic cleavage of membrane-bound SCF[111]. SCF binds and activates KIT, which is expressed at high levels by all LTR HSCs as well as other stem cells. Mutations at either of these loci affect migration and differentiation of primordial germ cells, neural-crest-derived melanoblasts, and haematopoietic cells[112] (see Supplementary information S1 (table)). Analysis of the different SCF and KIT mutant mice showed that although not essential for the generation and initial clonal expansion of HSCs in the embryo and fetal liver, they are crucial for long-term maintenance and self-renewal of adult HSCs, raising the possibility that the SCF–KIT pathway mediates endosteal bone-marrow HSC niche activity (FIG. 5).

Angiogenic factors
These factors (which include angiopoietin-1) promote the development of blood vessels, and are particularly important in embryonic and fetal development.

Importantly, membrane-bound SCF is expressed by osteoblasts and has a higher and more sustained capacity to activate KIT on the cell surface of HSCs than secreted SCF[112,113]. In addition, membrane-bound SCF is a potent stimulator of adhesion of HSCs or haematopoietic progenitor cells to stromal cells[114] because it can activate VLA4 and VLA5, indicating that membrane-bound SCF can affect the adhesive properties of the endosteal niche by modifying the functional state of specific integrins[115]. Transplantation of normal bone marrow into *Sl/Sl*^{d} mice results in impaired lodging and engraftment of the transplanted HSCs[12,116]. In addition, the bone marrow of young *Sl/Sl*^{d} mice has normal LTR activity when transplanted into lethally irradiated recipients, whereas bone marrow from old *Sl/Sl*^{d} mice has greatly reduced LTR activity, indicating a progressive loss of HSC activity over time, potentially due to ceasing niche activity[13,14]. Collectively, these data indicate that membrane-bound SCF is an essential component of the endosteal bone-marrow HSC niche that maintains long-term HSC activity in adult bone marrow. However, membrane-bound SCF is also required for osteoblast proliferation and activity *in vivo*, as shown by the development of osteopaenia in *Sl/Sl*^{d} mice[117] (TABLE 1). Therefore, further research is required to clarify whether the effect of membrane-bound SCF is direct (due to its capacity to provide sustained activation of KIT expressed by HSCs), or whether it is indirect (owing to its essential role in the maintenance of niche osteoblasts).

N-cadherin: a central HSC anchor? N-cadherin is expressed by both SNOs and a subset of LSK HSCs[39,52]. In addition, N-cadherin expression by HSCs localizes asymmetrically to the side of their attachment to SNOs[39].

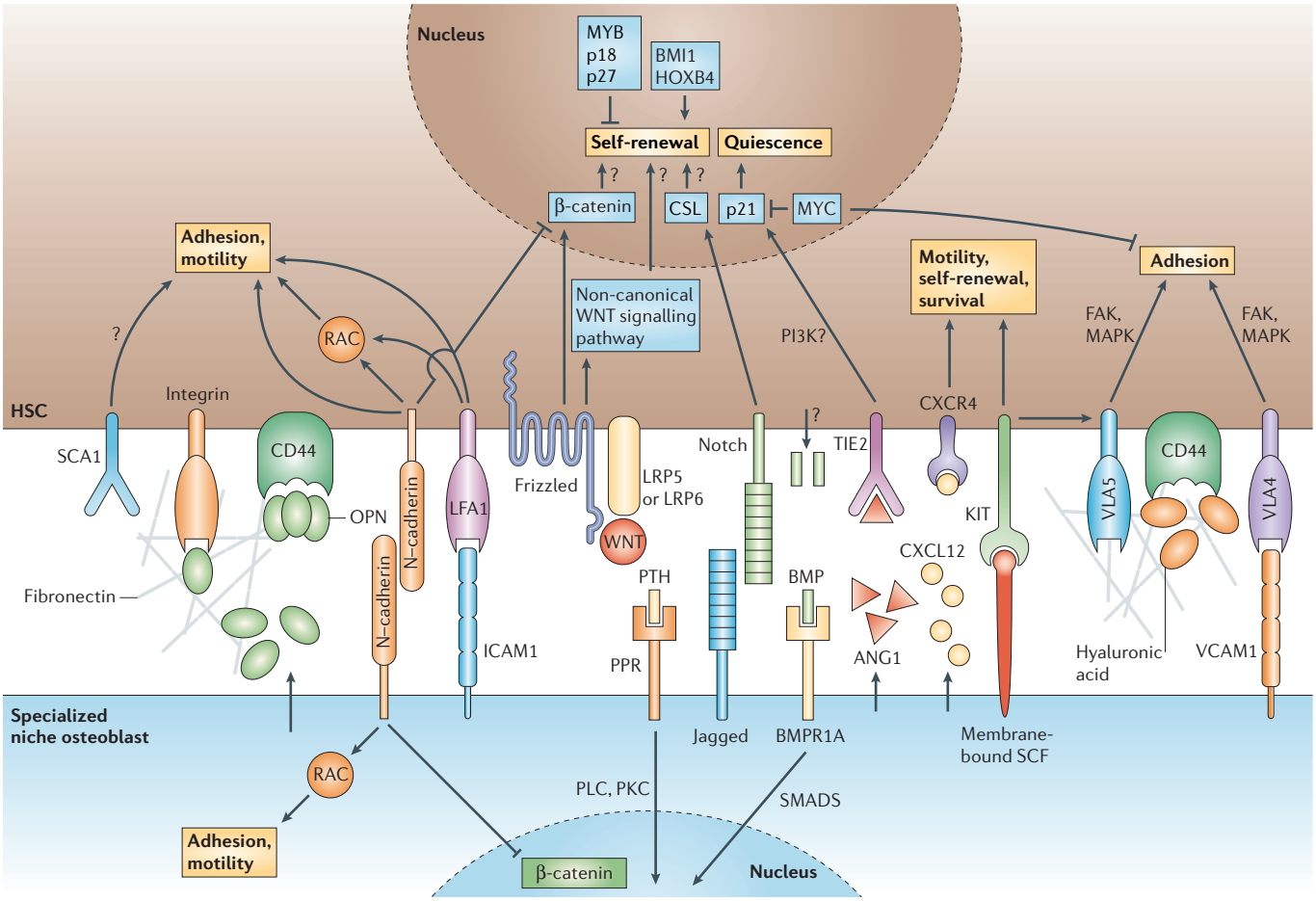


Figure 5 | **A model of the endosteal niche–stem-cell synapse.** Schematic diagram of the endosteal niche–stem-cell synapse showing putative ligand–receptor interactions and adhesion molecules, as well as some of the intracellular pathways that are activated following signalling. ANG1, angiopoietin-1; BMI1, polycomb repressor; BMP, bone morphogenetic protein; BMPR1A, BMP receptor 1A; CSL, CBF1 suppressor of Hairless and LAG1; CXCL12, CXC-chemokine ligand 12; CXCR4, CXC-chemokine receptor 4; FAK, focal adhesion kinase; HOXB4, homeobox B4; HSC, haematopoietic stem cell; ICAM1, intercellular adhesion molecule 1; LFA1, lymphocyte function-associated antigen 1; LRP, low-density-lipoprotein-receptor-related protein; MAPK, mitogen-activated protein kinase; OPN, osteopontin; PI3K, phosphatidylinositol-3 kinase; PLC, phospholipase C; PKC, protein kinase C; PPR, PTH/PTH-related protein receptor; PTH, parathyroid hormone; SCF, stem-cell factor; SMADS, mothers against decapentaplegic-related homologue; SNO, spindle-shaped N-cadherin-expressing osteoblast; TIE2, tyrosine kinase receptor 2; VCAM1, vascular cell-adhesion molecule 1; VLA4, very late antigen 4; '?' denotes molecules and/or interactions for which only indirect or contradictory evidence is available.

Therefore, homotypic N-cadherin interactions have been postulated to be an important component of the anchor that links HSCs to SNOs in the endosteal niche.

In support of this hypothesis, ectopic expression of N-cadherin by OP9 stromal cells substantially increases their ability to maintain mouse HSCs *in vitro*[45]. However, genetic evidence of an essential role for N-cadherin in HSC–osteoblast adhesion and/or signalling is still lacking, as N-cadherin-mutant embryos fail to develop past mid-gestation[118]. Moreover, whether functional HSCs are enriched in N-cadherin-expressing LSK HSCs, compared to those not expressing this adhesion receptor, has not been shown. Nevertheless, indirect support for the importance of N-cadherin has been obtained from studies showing that MYC and tyrosine kinase receptor 2 (TIE2) control N-cadherin expression by HSCs in an antagonistic manner. The effects of MYC and TIE2 on HSCs and on N-cadherin expression correlate with a key function for N-cadherin in the retention of HSCs in the endosteal niche[43,45,52,53].

The cell-adhesion signalling network. Genetic evidence for the requirement of TIE2 in HSC–niche interactions has been obtained from chimeric mice comprised of wild-type and *TIE1*$^{-/-}$*TIE2*$^{-/-}$ morulae[119]. Although TIE1 and TIE2 are not required for the development and differentiation of fetal HSCs, HSCs lacking both TIE1 and TIE2 fail to be maintained in the adult microenvironment. In adult bone marrow, TIE2 (which is expressed specifically by LT-HSCs) is activated by angiopoietin-1 (ANG1), which is secreted by osteoblasts, leading to upregulation of N-cadherin expression by HSCs, providing the first example of a secreted factor promoting HSC–osteoblast adhesion. Interestingly, the ANG1–TIE2 signalling pathway prevents HSC division and maintains HSC quiescence, both *in vitro* and *in vivo*[36,41,45,120]. Collectively, these data strongly support the hypothesis that N-cadherin-expressing ANG1^{+} osteoblasts form a niche that maintains quiescence and prevents self-renewal or differentiation through TIE2 signalling (FIG. 5).

TIE2-mediated quiescence is potentially caused by positively regulating the cyclin-dependent-kinase inhibitor p21 (also known as CIP1 and WAF1). HSCs express high levels of p21, and mice lacking p21 show increased HSC proliferation at the expense of long-term self-renewal, indicating that p21 is essential for maintenance of quiescence in HSCs[120,121] (see Supplementary information S1 (table)). In contrast to TIE2, transcription of the gene encoding p21 is negatively regulated by MYC, which is expressed at low levels by HSCs but increases during initiation of HSC differentiation in a converse expression pattern to that of p21 (REFS 52,121,122). Interestingly, MYC-deficient LSKFLT3^{-} HSCs overexpress N-cadherin and integrins such as lymphocyte function-associated antigen-1 (LFA1) and VLA5, and contact SNOs[52]. Although mutant LSKFLT3^{-} HSCs self-renew normally, they have a severe niche-dependent differentiation defect and accumulate *in situ*. Conversely, enforced MYC activity in HSCs represses the expression of N-cadherin, as well as the expression of several integrins, by LSK HSCs. Most importantly, MYC overexpressing HSCs are lost over time because of differentiation, presumably owing to their failure to be retained in the niche[52]. These data indicate that the balance between self-renewal and differentiation might be controlled by MYC-dependent retention or exit of HSCs from the niche[52,53].

N-cadherin and WNT signalling. Intriguingly, it has recently been shown that the transmembrane metalloproteinase ADAM10 (a disintegrin and metalloproteinase-10) is able to cleave N-cadherin that is expressed at the cell surface of fibroblasts and neuronal cells. This leads to the redistribution of β-catenin (which is associated with the intracellular portion of N-cadherin) from the cell surface to the cytoplasmic β-catenin pool, thereby decreasing the signalling threshold required for the expression of target genes of the canonical WNT signalling pathway (which is mediated through β-catenin signal transduction cascades), such as the genes encoding cyclin D1 and MYC[123]. A similar re-distribution of β-catenin has also been reported after E-cadherin cleavage[124], indicating that high levels of expression of cadherins, as observed for HSCs, might decrease cytoplasmic β-catenin levels and therefore negatively regulate expression of β-catenin target genes.

This contrasts with studies in which activation of the WNT signalling pathway in cultured HSCs promotes symmetrical self-renewal in the absence of differentiation[125,126]. However, the importance of canonical WNT signalling during haematopoiesis has recently been questioned because β-catenin is dispensable for HSC function[127]. Although it is probable that the WNT signalling pathway has an important role in HSC or haematopoietic progenitor cell function[100], the question remains whether WNT promotes self-renewal of LT-HSCs *in vivo*, or whether it is only important for the expansion and differentiation of non-HSC haematopoietic progenitor cells. The latter is in agreement with the expression pattern of the β-catenin target gene *Myc*, which is induced in LSKFLT3^{+} progenitor cells leading to downregulation of N-cadherin and integrin expression[52]. In this context, it is intriguing that the N-cadherin, TIE2, MYC, p21 and β-catenin pathways are apparently interconnected, leading to the suggestion that they might cooperatively control quiescence, self-renewal and initiation of HSC differentiation through interaction with the niche[53] (FIG. 5 and Supplementary information S1 (table)).

The stem-cell–niche synapse

The picture emerging from accumulating genetic and functional data indicates that molecular crosstalk between HSCs and niche cells (particularly osteoblasts) involves a large number of molecules (cadherins, integrins, chemokines, cytokines, signalling molecules and receptors) that mediate at least two types of interaction (FIG. 5). First, adhesive cell–extracellular-matrix (ECM) interactions such as CD44 binding to OPN or hyaluronic acid, and cell–cell interactions, such as those mediated by heterotypic VLA4–VCAM1 interactions and homotypic N-cadherin interactions. The main function of these interactions would be to maintain HSCs in close

OP9 stromal cells
A bone-marrow-derived cell line that can support the expansion of haematopoietic-cell lineages in culture.

proximity to cells in the endosteal bone-marrow niche. In addition, most adhesion receptors are also linked to intracellular signalling cascades and actively participate in the signalling network controlling HSC maintenance (FIG. 5). Second, ligand–receptor interactions, through which intracellular signalling pathways are activated after ligand binding to receptors that are expressed by HSCs or SNOs (FIG. 5).

Most secreted signalling molecules are bound to the cell surface or ECM, and consequently do not diffuse far. Therefore, the tight adhesion and juxtaposition of HSCs to niche osteoblasts is essential for the formation of an intercellular space in which efficient ligand–receptor interaction can occur. Some osteoblast-derived signals might be crucial to maintain HSCs in an undifferentiated state and these include the ligand–receptor pairs membrane-bound SCF–KIT, and ANG1–TIE2–MYC (see Supplementary information S1 (table)). Conversely, other ligand–receptor pairs, such as BMP–BMPR1A are important for the number and/or activity of niche osteoblasts. Therefore, in analogy to the neuronal and immunological synapses[128], we propose the term 'stem-cell–niche synapse' for this adhesion and signalling unit (FIG. 5).

Concluding remarks

During the past few years, the theoretical concept of a specific stem-cell microenvironment (that is, a stem-cell niche) that was proposed in the 1960s and 1970s, has finally received greater attention[129]. Substantial progress in localizing the bone-marrow HSC niche(s), as well as its characterization at the molecular and cellular levels, has been made. Nevertheless, important questions remain. These include, how many different types of haematopoietic niches exist in the bone marrow and the periphery, how many HSCs each niche contains, and the exact role each niche unit has during homeostasis and in response to bone-marrow stress. It also remains unclear whether HSC–niche interactions are stable or dynamic. Furthermore, although osteoblasts have been shown to be rate-limiting for HSC number, very little is known about the specific differentiation stage of these cells. Are they the same mesenchymal-stem-cell-derived osteoblasts that continue to differentiate into osteocytes, or have they branched off to generate a distinct 'niche-osteoblast'? If the latter is the case, do they differentiate in response to signals that are derived from an attaching HSC? The recently identified vascular niche[9] opens another chapter on HSC–bone-marrow-niche interactions, and the molecular events governing adhesion and signalling of BMECs with HSCs will be an area of intense future research and will move the endothelial-cell field to one of the centre stages of adult-stem-cell research.

Finally, whether long-term self-renewal occurs in sites of extramedullary haematopoiesis, such as the spleen and liver, and therefore maintains blood formation during acute and chronic bone-marrow injury, remains unclear. First attempts to address this question indicate that mobilized splenic HSCs are found close to the vasculature (peripheral vascular niche)[9]. Are these areas active niches or do they only transiently maintain HSCs? Are they always present or do they form only after injury, and what are the equivalent niche structures in the liver? At the moment there are more questions than answers, but a better understanding of the different niches will also unearth similarities between them, which should facilitate the eventual reconstruction of active niches *in vitro*.

Collectively, the impressive progress in the HSC-niche field clearly indicates that substantial clonal expansion of HSCs *in vitro* unquestionably requires more than just a cytokine cocktail, and instead requires a three-dimensional reconstruction of the niche, including the appropriate cells and ECM to allow the generation of a stable 'stem-cell–niche synapse'. This requires not only further progress from the cell–and-molecular-biology end, but is in urgent need of input from matrix- and tissue-engineering fields. Future perspectives have never been more promising, and a breakthrough in the *in vitro* expansion field will eliminate one of the main obstacles for future regenerative medicine using adult stem cells[130].

Note added in proof

A recent report shows that HSCs that are deficient for the calcium-sensing receptor show decreased homing to the endosteal niche accompanied by diminished adhesion to collagen type I. These data indicate that local calcium gradients, as are observed around areas of bone remodelling, might be involved in engraftment and/or retention of HSCs to the endosteal niche[141].

1. Fuchs, E., Tumbar, T. & Guasch, G. Socializing with the neighbors: stem cells and their niche. *Cell* **116**, 769–778 (2004).
2. Osawa, M., Hanada, K., Hamada, H. & Nakauchi, H. Long-term lymphohematopoietic reconstitution by a single CD34$^{-low/negative}$ hematopoietic stem cell. *Science* **273**, 242–245 (1996).
3. Watt, F. M. & Hogan, B. L. Out of Eden: stem cells and their niches. *Science* **287**, 1427–1430 (2000).
4. Weissman, I. L. Stem cells: units of development, units of regeneration, and units in evolution. *Cell* **100**, 157–168 (2000).
5. Kondo, M. *et al*. Biology of hematopoietic stem cells and progenitors: implications for clinical application. *Annu. Rev. Immunol.* **21**, 759–806 (2003).
6. Till, J. E. & McCulloch, C. E. A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. *Radiat. Res.* **14**, 213–222 (1961).
7. Wagers, A. J., Sherwood, R. I., Christensen, J. L. & Weissman, I. L. Little evidence for developmental plasticity of adult hematopoietic stem cells. *Science* **297**, 2256–2259 (2002).
8. Matsuzaki, Y., Kinjo, K., Mulligan, R. C. & Okano, H. Unexpectedly efficient homing capacity of purified murine hematopoietic stem cells. *Immunity* **20**, 87–93 (2004).
9. Kiel, M. J., Yilmaz, O. H., Iwashita, T., Terhorst, C. & Morrison, S. J. SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. *Cell* **121**, 1109–1121 (2005).
This study shows that CD150, one of the signalling lymphocytic activation molecules (SLAMs) is expressed by LTR HSCs. CD150^{+} HSCs were localized near sinusoids in normal bone marrow, indicating that in addition to the endosteal niche a second vascular niche might exist.
10. Curry, J. L., Trentin, J. J. & Wolf, N. Hemopoietic spleen colony studies. II. Erythropoiesis. *J. Exp. Med.* **125**, 703–720 (1967).
11. Schofield, R. The relationship between the spleen colony-forming cell and the haemopoietic stem cell. *Blood Cells* **4**, 7–25 (1978).
This paper proposes that HSCs are associated with other cell types, and that these allow self-renewal but prevent cell maturation. Schofield termed this microenvironment the 'stem-cell niche'.
12. McCulloch, E. A., Siminovitch, L., Till, J. E., Russell, E. S. & Bernstein, S. E. The cellular basis of the genetically determined hemopoietic defect in anemic mice of genotype *Sl-Sl^{d}*. *Blood* **26**, 399–410 (1965).

This paper (together with references 13 and 14) shows that normal bone marrow fails to engraft in *Sl/Sl*^{d} mice. This is probably the first study demonstrating that the microenvironment is essential for bone-marrow HSC function and/or maintenance.

13. Barker, J. E. Sl/Sld hematopoietic progenitors are deficient *in situ*. *Exp. Hematol.* **22**, 174–177 (1994).
14. Barker, J. E. Early transplantation to a normal microenvironment prevents the development of *Steel* hematopoietic stem cell defects. *Exp. Hematol.* **25**, 542–547 (1997).
15. Nilsson, S. K. & Simmons, P. J. Transplantable stem cells: home to specific niches. *Curr. Opin. Hematol.* **11**, 102–106 (2004).
16. Ohlstein, B., Kai, T., Decotto, E. & Spradling, A. The stem cell niche: theme and variations. *Curr. Opin. Cell Biol.* **16**, 693–699 (2004).
17. Spradling, A., Drummond-Barbosa, D. & Kai, T. Stem cells find their niche. *Nature* **414**, 98–104 (2001).
18. Wu, A. M., Siminovitch, L., Till, J. E. & McCulloch, E. A. Evidence for a relationship between mouse hemopoietic stem cells and cells forming colonies in culture. *Proc. Natl Acad. Sci. USA* **59**, 1209–1215 (1968).
19. Domen, J. & Weissman, I. L. Self-renewal, differentiation or death: regulation and manipulation of hematopoietic stem cell fate. *Mol. Med. Today* **5**, 201–208 (1999).
20. Yang, L. *et al*. Identification of Lin^{-}Sca1^{+}kit^{+}CD34^{+}Flt3^{-} short-term hematopoietic stem cells capable of rapidly reconstituting and rescuing myeloablated transplant recipients. *Blood* **105**, 2717–2723 (2005).
21. Adolfsson, J. *et al*. Identification of Flt3^{+} lympho-myeloid stem cells lacking erythro-megakaryocytic potential a revised road map for adult blood lineage commitment. *Cell* **121**, 295–306 (2005).
22. Christensen, J. L. & Weissman, I. L. Flk-2 is a marker in hematopoietic stem cell differentiation: a simple method to isolate long-term stem cells. *Proc. Natl Acad. Sci. USA* **98**, 14541–14546 (2001).
23. Jordan, C. T. & Lemischka, I. R. Clonal and systemic analysis of long-term hematopoiesis in the mouse. *Genes Dev.* **4**, 220–232 (1990).
24. Ramalho-Santos, M., Yoon, S., Matsuzaki, Y., Mulligan, R. C. & Melton, D. A. 'Stemness': transcriptional profiling of embryonic and adult stem cells. *Science* **298**, 597–600 (2002).
25. Ivanova, N. B. *et al*. A stem cell molecular signature. *Science* **298**, 601–604 (2002).
26. Akashi, K. *et al*. Transcriptional accessibility for genes of multiple tissues and hematopoietic lineages is hierarchically controlled during early hematopoiesis. *Blood* **101**, 383–389 (2003).
27. Venezia, T. A. *et al*. Molecular signatures of proliferation and quiescence in hematopoietic stem cells. *PLoS Biol.* **2**, e301 (2004).
28. Roegiers, F. & Jan, Y. N. Asymmetric cell division. *Curr. Opin. Cell Biol.* **16**, 195–205 (2004).
29. Betschinger, J. & Knoblich, J. A. Dare to be different: asymmetric cell division in *Drosophila*, *C. elegans* and vertebrates. *Curr. Biol.* **14**, R674–R685 (2004).
30. Lechler, T. & Fuchs, E. Asymmetric cell divisions promote stratification and differentiation of mammalian skin. *Nature* **437**, 275–280 (2005).
This paper shows that some basal cells of the skin epidermis divide in an asymmetric manner (divisional asymmetry), and that this correlates with the unequal distribution of determinants including PAR3, LGN and mINSC in the two daughter cells.
31. Suda, J., Suda, T. & Ogawa, M. Analysis of differentiation of mouse hemopoietic stem cells in culture by sequential replating of paired progenitors. *Blood* **64**, 393–399 (1984).
32. Takano, H., Ema, H., Sudo, K. & Nakauchi, H. Asymmetric division and lineage commitment at the level of hematopoietic stem cells: inference from differentiation in daughter cell and granddaughter cell pairs. *J. Exp. Med.* **199**, 295–302 (2004).
33. Ho, A. D. Kinetics and symmetry of divisions of hematopoietic stem cells. *Exp. Hematol.* **33**, 1–8 (2005).
34. Xie, T. & Spradling, A. C. A niche maintaining germ line stem cells in the *Drosophila* ovary. *Science* **290**, 328–330 (2000).
35. Kiger, A. A., White-Cooper, H. & Fuller, M. T. Somatic support cells restrict germline stem cell self-renewal and promote differentiation. *Nature* **407**, 750–754 (2000).
36. Fleming, W. H. *et al*. Functional heterogeneity is associated with the cell cycle status of murine hematopoietic stem cells. *J. Cell Biol.* **122**, 897–902 (1993).
37. Cotsarelis, G., Sun, T. T. & Lavker, R. M. Label-retaining cells reside in the bulge area of pilosebaceous unit: implications for follicular stem cells, hair cycle, and skin carcinogenesis. *Cell* **61**, 1329–1337 (1990).
38. Tumbar, T. *et al*. Defining the epithelial stem cell niche in skin. *Science* **303**, 359–363 (2004).
In this paper, transgenic expression of H2B–EGFP is used to show that slow-cycling skin epidermal stem cells are primarily located in the hair follicle bulge. This technique, which identifies and allows purification of viable LRCs, should be adaptable for analysis of other adult stem cells and their associated niches.
39. Zhang, J. *et al*. Identification of the haematopoietic stem cell niche and control of the niche size. *Nature* **425**, 836–841 (2003).
N-cadherin was proposed as a critical molecule that anchors HSCs to osteoblasts through homotypic interactions. This study (together with reference 67), also shows that osteoblastic cells are a critical component of the endosteal bone-marrow niche.
40. Lerner, C. & Harrison, D. E. 5-Fluorouracil spares hemopoietic stem cells responsible for long-term repopulation. *Exp. Hematol.* **18**, 114–118 (1990).
41. Spangrude, G. J. & Johnson, G. R. Resting and activated subsets of mouse multipotent hematopoietic stem cells. *Proc. Natl Acad. Sci. USA* **87**, 7433–7437 (1990).
42. Uchida, N. *et al*. The unexpected G_0/G_1 cell cycle status of mobilized hematopoietic stem cells from peripheral blood. *Blood* **89**, 465–472 (1997).
43. Suda, T., Arai, F. & Hirao, A. Hematopoietic stem cells and their niche. *Trends Immunol.* **26**, 426–433 (2005).
44. Potten, C. S. & Loeffler, M. Stem cells: attributes, cycles, spirals, pitfalls and uncertainties. Lessons for and from the crypt. *Development* **110**, 1001–1020 (1990).
45. Arai, F. *et al*. Tie2/angiopoietin-1 signalling regulates hematopoietic stem cell quiescence in the bone marrow niche. *Cell* **118**, 149–161 (2004).
This paper shows that the ANG1 produced by niche osteoblasts activates TIE2, which maintains HSC quiescence and increases N-cadherin expression, and therefore increases adhesion to the endosteal niche.
46. Oshima, H., Rochat, A., Kedzia, C., Kobayashi, K. & Barrandon, Y. Morphogenesis and renewal of hair follicles from adult multipotent stem cells. *Cell* **104**, 233–245 (2001).
47. Van Zant, G. Studies of hematopoietic stem cells spared by 5-fluorouracil. *J. Exp. Med.* **159**, 679–690 (1984).
48. Randall, T. D. & Weissman, I. L. Phenotypic and functional changes induced at the clonal level in hematopoietic stem cells after 5-fluorouracil treatment. *Blood* **89**, 3596–3606 (1997).
49. Uchida, N. *et al*. ABC transporter activities of murine hematopoietic stem cells vary according to their developmental and activation status. *Blood* **103**, 4487–4495 (2004).
50. Chambers, I. & Smith, A. Self-renewal of teratocarcinoma and embryonic stem cells. *Oncogene* **23**, 7150–7160 (2004).
51. Cheshier, S. H., Morrison, S. J., Liao, X. & Weissman, I. L. *In vivo* proliferation and cell cycle kinetics of long-term self- renewing hematopoietic stem cells. *Proc. Natl Acad. Sci. USA* **96**, 3120–3125 (1999).
52. Wilson, A. *et al*. c-Myc controls the balance between hematopoietic stem cell self-renewal and differentiation. *Genes Dev.* **18**, 2747–2763 (2004).
This paper provides genetic evidence that differential expression of MYC controls the balance between stem-cell self-renewal and differentiation by regulating entry and exit of HSCs from the endosteal niche.
53. Murphy, M. J., Wilson, A. & Trumpp, A. More than just proliferation: Myc function in stem cells. *Trends Cell Biol.* **15**, 128–137 (2005).
54. Patt, H. M. & Maloney, M. A. Bone formation and resorption as a requirement for marrow development. *Proc. Soc. Exp. Biol. Med.* **140**, 205–207 (1972).
55. Maloney, M. A. & Patt, H. M. On the origin of hematopoietic stem cells after local marrow extirpation. *Proc. Soc. Exp. Biol. Med.* **149**, 94–97 (1975).
56. Deguchi, K. *et al*. Excessive extramedullary hematopoiesis in Cbfa1-deficient mice with a congenital lack of bone marrow. *Biochem. Biophys. Res. Commun.* **255**, 352–359 (1999).
57. Ducy, P., Schinke, T. & Karsenty, G. The osteoblast: a sophisticated fibroblast under central surveillance. *Science* **289**, 1501–1504 (2000).
58. Karsenty, G. & Wagner, E. F. Reaching a genetic and molecular understanding of skeletal development. *Dev. Cell* **2**, 389–406 (2002).
59. Lord, B. I., Testa, N. G. & Hendry, J. H. The relative spatial distributions of CFUs and CFUc in the normal mouse femur. *Blood* **46**, 65–72 (1975).
60. Gong, J. K. Endosteal marrow: a rich source of hematopoietic stem cells. *Science* **199**, 1443–1445 (1978).
This study provides the first functional evidence for the location of HSCs and progenitor cells close to endosteal surfaces of rat bone.
61. Askenasy, N. & Farkas, D. L. *In vivo* imaging studies of the effect of recipient conditioning, donor cell phenotype and antigen disparity on homing of haematopoietic cells to the bone marrow. *Br. J. Haematol.* **120**, 505–515 (2003).
62. Sipkins, D. A. *et al*. *In vivo* imaging of specialized bone marrow endothelial microdomains for tumour engraftment. *Nature* **435**, 969–973 (2005).
63. Nilsson, S. K., Johnston, H. M. & Coverdale, J. A. Spatial localization of transplanted hemopoietic stem cells: inferences for the localization of stem cell niches. *Blood* **97**, 2293–2299 (2001).
64. Taichman, R. S. & Emerson, S. G. The role of osteoblasts in the hematopoietic microenvironment. *Stem Cells* **16**, 7–15 (1998).
65. Taichman, R. S. Blood and bone: two tissues whose fates are intertwined to create the hematopoietic stem-cell niche. *Blood* **105**, 2631–2639 (2005).
66. Oostendorp, R. A. *et al*. Stromal cell lines from mouse aorta-gonads-mesonephros subregions are potent supporters of hematopoietic stem cell activity. *Blood* **99**, 1183–1189 (2002).
67. Calvi, L. M. *et al*. Osteoblastic cells regulate the haematopoietic stem cell niche. *Nature* **425**, 841–846 (2003).
68. Chen, D., Zhao, M. & Mundy, G. R. Bone morphogenetic proteins. *Growth Factors* **22**, 233–241 (2004).
69. Visnjic, D. *et al*. Conditional ablation of the osteoblast lineage in Col2.3δk transgenic mice. *J. Bone Miner. Res.* **16**, 2222–2231 (2001).
70. Visnjic, D. *et al*. Hematopoiesis is severely altered in mice with an induced osteoblast deficiency. *Blood* **103**, 3258–3264 (2004).
This paper shows that conditional ablation of osteoblasts results in a reversible decrease of bone-marrow HSCs and extramedullary haematopoiesis, indicating that osteoblasts are not only required for maintenance of bone-marrow haematopoiesis, but are also an essential component of the niche.
71. Corral, D. A. *et al*. Dissociation between bone resorption and bone formation in osteopenic transgenic mice. *Proc. Natl Acad. Sci. USA* **95**, 13835–13840 (1998).
72. Huber, T. L., Kouskoff, V., Fehling, H. J., Palis, J. & Keller, G. Haemangioblast commitment is initiated in the primitive streak of the mouse embryo. *Nature* **432**, 625–630 (2004).
73. Ohneda, O. *et al*. Hematopoietic stem cell maintenance and differentiation are supported by embryonic aorta-gonad-mesonephros region-derived endothelium. *Blood* **92**, 908–919 (1998).
74. Li, W. *et al*. Primary endothelial cells isolated from the yolk sac and para-aortic splanchnopleura support the expansion of adult marrow stem cells *in vitro*. *Blood* **102**, 4345–4353 (2003).
75. Li, W., Johnson, S. A., Shelley, W. C. & Yoder, M. C. Hematopoietic stem cell repopulating ability can be maintained *in vitro* by some primary endothelial cells. *Exp. Hematol.* **32**, 1226–1237 (2004).
76. Kopp, H. G., Avecilla, S. T., Hooper, A. T. & Rafii, S. The bone marrow vascular niche: home of HSC differentiation and mobilization. *Physiology (Bethesda Md.)* **20**, 349–356 (2005).
77. Avecilla, S. T. *et al*. Chemokine-mediated interaction of hematopoietic progenitors with the bone marrow vascular niche is required for thrombopoiesis. *Nature Med.* **10**, 64–71 (2004).
78. Rafii, S., Mohle, R., Shapiro, F., Frey, B. M. & Moore, M. A. Regulation of hematopoiesis by microvascular endothelium. *Leuk. Lymphoma* **27**, 375–386 (1997).

79. Heissig, B. *et al*. Recruitment of stem and progenitor cells from the bone marrow niche requires MMP-9 mediated release of kit-ligand. *Cell* **109**, 625–637 (2002).
80. Rafii, S. *et al*. Human bone marrow microvascular endothelial cells support long-term proliferation and differentiation of myeloid and megakaryocytic progenitors. *Blood* **86**, 3353–3363 (1995).
81. Delehanty, L. L. *et al*. Stromal inhibition of megakaryocytic differentiation is associated with blockade of sustained Rap1 activation. *Blood* **101**, 1744–1751 (2003).
82. Tokoyoda, K., Egawa, T., Sugiyama, T., Choi, B. I. & Nagasawa, T. Cellular niches controlling B lymphocyte behaviour within bone marrow during development. *Immunity* **20**, 707–718 (2004).
83. Hirose, J. *et al*. A developing picture of lymphopoiesis in bone marrow. *Immunol. Rev.* **189**, 28–40 (2002).
84. Papayannopoulou, T. Bone marrow homing: the players, the playfield, and their evolving roles. *Curr. Opin. Hematol.* **10**, 214–219 (2003).
85. Lapidot, T., Dar, A. & Kollet, O. How do stem cells find their way home? *Blood* **106**, 1901–1910 (2005).
86. Cancelas, J. A. *et al*. Rac GTPases differentially integrate signals regulating hematopoietic stem cell localization. *Nature Med.* **11**, 886–891 (2005).
87. Lapidot, T. & Petit, I. Current understanding of stem cell mobilization: the roles of chemokines, proteolytic enzymes, adhesion molecules, cytokines, and stromal cells. *Exp. Hematol.* **30**, 973–981 (2002).
88. Wright, D. E., Wagers, A. J., Gulati, A. P., Johnson, F. L. & Weissman, I. L. Physiological migration of hematopoietic stem and progenitor cells. *Science* **294**, 1933–1936 (2001).
89. Potocnik, A. J., Brakebusch, C. & Fassler, R. Fetal and adult hematopoietic stem cells require β_1 integrin function for colonizing fetal liver, spleen, and bone marrow. *Immunity* **12**, 653–663 (2000).
90. Nilsson, S. K. *et al*. Hyaluronan is synthesized by primitive hemopoietic cells, participates in their lodgment at the endosteum following transplantation, and is involved in the regulation of their proliferation and differentiation *in vitro*. *Blood* **101**, 856–862 (2003).
91. Nilsson, S. K. *et al*. Osteopontin, a key component of the hematopoietic stem cell niche and regulator of primitive hematopoietic progenitor cells. *Blood* **106**, 1232–1239 (2005).
92. Ara, T. *et al*. Long-term hematopoietic stem cells require stromal cell-derived factor-1 for colonizing bone marrow during ontogeny. *Immunity* **19**, 257–267 (2003).
The authors show that CXCL12 has an important role in colonization of the bone marrow by HSCs.
93. Ponomaryov, T. *et al*. Induction of the chemokine stromal-derived factor-1 following DNA damage improves human stem cell function. *J. Clin. Invest.* **106**, 1331–1339 (2000).
94. Wright, D. E., Bowman, E. P., Wagers, A. J., Butcher, E. C. & Weissman, I. L. Hematopoietic stem cells are uniquely selective in their migratory response to chemokines. *J. Exp. Med.* **195**, 1145–1154 (2002).
95. Nagasawa, T. *et al*. Defects of B-cell lymphopoiesis and bone-marrow myelopoiesis in mice lacking the CXC chemokine PBSF/SDF-1. *Nature* **382**, 635–638 (1996).
96. Zou, Y. R., Kottmann, A. H., Kuroda, M., Taniuchi, I. & Littman, D. R. Function of the chemokine receptor CXCR4 in haematopoiesis and in cerebellar development. *Nature* **393**, 595–599 (1998).
97. Gu, Y. *et al*. Hematopoietic cell regulation by Rac1 and Rac2 guanosine triphosphatases. *Science* **302**, 445–449 (2003).
98. Moore, K. A. Recent advances in defining the hematopoietic stem cell niche. *Curr. Opin. Hematol.* **11**, 107–111 (2004).
99. Artavanis-Tsakonas, S., Rand, M. D. & Lake, R. J. Notch signalling: cell fate control and signal integration in development. *Science* **284**, 770–776 (1999).
100. Reya, T. & Clevers, H. Wnt signalling in stem cells and cancer. *Nature* **434**, 843–850 (2005).
101. Radtke, F., Wilson, A., Mancini, S. J. & MacDonald, H. R. Notch regulation of lymphocyte development and function. *Nature Immunol.* **5**, 247–253 (2004).
102. Duncan, A. W. *et al*. Integration of Notch and Wnt signalling in hematopoietic stem cell maintenance. *Nature Immunol.* **6**, 314–322 (2005).
103. Varnum-Finney, B. *et al*. Pluripotent, cytokine-dependent, hematopoietic stem cells are immortalized by constitutive Notch1 signalling. *Nature Med.* **6**, 1278–1281 (2000).
104. Stier, S., Cheng, T., Dombkowski, D., Carlesso, N. & Scadden, D. T. Notch1 activation increases hematopoietic stem cell self-renewal *in vivo* and favours lymphoid over myeloid lineage outcome. *Blood* **99**, 2369–2378 (2002).
105. Han, H. *et al*. Inducible gene knockout of transcription factor recombination signal binding protein-J reveals its essential role in T versus B lineage decision. *Int. Immunol.* **14**, 637–645 (2002).
106. Mancini, S. J. *et al*. Jagged1-dependent Notch signalling is dispensable for hematopoietic stem cell self-renewal and differentiation. *Blood* **105**, 2340–2342 (2005).
107. Radtke, F. *et al*. Deficient T cell fate specification in mice with an induced inactivation of Notch1. *Immunity* **10**, 547–558 (1999).
108. Saito, T. *et al*. Notch2 is preferentially expressed in mature B cells and indispensable for marginal zone B lineage development. *Immunity* **18**, 675–685 (2003).
109. Denhardt, D. T. & Guo, X. Osteopontin: a protein with diverse functions. *FASEB J.* **7**, 1475–1482 (1993).
110. Stier, S. *et al*. Osteopontin is a hematopoietic stem cell niche component that negatively regulates stem cell pool size. *J. Exp. Med.* **201**, 1781–1791 (2005).
111. Flanagan, J. G., Chan, D. C. & Leder, P. Transmembrane form of the kit ligand growth factor is determined by alternative splicing and is missing in the Sld mutant. *Cell* **64**, 1025–1035 (1991).
112. Lyman, S. D. & Jacobsen, S. E. c-kit ligand and Flt3 ligand: stem/progenitor cell factors with overlapping yet distinct activities. *Blood* **91**, 1101–1134 (1998).
113. Miyazawa, K. *et al*. Membrane-bound Steel factor induces more persistent tyrosine kinase activation and longer life span of c-kit gene-encoded protein than its soluble form. *Blood* **85**, 641–649 (1995).
114. Kinashi, T. & Springer, T. A. Steel factor and c-kit regulate cell-matrix adhesion. *Blood* **83**, 1033–1038 (1994).
115. Kovach, N. L., Lin, N., Yednock, T., Harlan, J. M. & Broudy, V. C. Stem cell factor modulates avidity of α_4 β_1 and α_5 β_1 integrins expressed on hematopoietic cell lines. *Blood* **85**, 159–167 (1995).
116. Driessen, R. L., Johnston, H. M. & Nilsson, S. K. Membrane-bound stem cell factor is a key regulator in the initial lodgment of stem cells within the endosteal marrow region. *Exp. Hematol.* **31**, 1284–1291 (2003).
117. Lotinun, S., Evans, G. L., Turner, R. T. & Oursler, M. J. Deletion of membrane-bound steel factor results in osteopenia in mice. *J. Bone Miner. Res.* **20**, 644–652 (2005).
118. Radice, G. L. *et al*. Developmental defects in mouse embryos lacking N-cadherin. *Dev. Biol.* **181**, 64–78 (1997).
119. Puri, M. C. & Bernstein, A. Requirement for the TIE family of receptor tyrosine kinases in adult but not fetal hematopoiesis. *Proc. Natl Acad. Sci. USA* **100**, 12753–12758 (2003).
120. Cheng, T. *et al*. Hematopoietic stem cell quiescence maintained by *p21cip1/waf1*. *Science* **287**, 1804–1808 (2000).
121. Cheng, T., Shen, H., Rodrigues, N., Stier, S. & Scadden, D. T. Transforming growth factor β1 mediates cell-cycle arrest of primitive hematopoietic cells independent of p21(Cip1/Waf1) or p27(Kip1). *Blood* **98**, 3643–3649 (2001).
122. Wu, S. *et al*. Myc represses differentiation-induced p21CIP1 expression via Miz-1-dependent interaction with the p21 core promoter. *Oncogene* **22**, 351–360 (2003).
123. Reiss, K. *et al*. ADAM10 cleavage of N-cadherin and regulation of cell–cell adhesion and β-catenin nuclear signalling. *EMBO J.* **24**, 742–752 (2005).
124. Ito, K. *et al*. Calcium influx triggers the sequential proteolysis of extracellular and cytoplasmic domains of E-cadherin, leading to loss of β-catenin from cell–cell contacts. *Oncogene* **18**, 7080–7090 (1999).
125. Willert, K. *et al*. Wnt proteins are lipid-modified and can act as stem cell growth factors. *Nature* **423**, 448–452 (2003).
This paper, together with reference 126, provides evidence of a positive role for WNT signalling in HSC self-renewal, whereas differentiation (at least *in vitro*) is inhibited.
126. Reya, T. *et al*. A role for Wnt signalling in self-renewal of haematopoietic stem cells. *Nature* **423**, 409–414 (2003).
127. Cobas, M. *et al*. β-catenin is dispensable for hematopoiesis and lymphopoiesis. *J. Exp. Med.* **199**, 221–229 (2004).
128. Friedl, P. & Storim, J. Diversity in immune-cell interactions: states and functions of the immunological synapse. *Trends Cell Biol.* **14**, 557–567 (2004).
129. Powell, K. It's the ecology, stupid. *Nature* **435**, 268–270 (2005).
130. Sorrentino, B. P. Clinical strategies for expansion of haematopoietic stem cells. *Nature Rev. Immunol.* **4**, 878–888 (2004).
131. Ploemacher, R. E. Stem cells: characterization and measurement. *Baillieres Clin. Haematol.* **10**, 429–444 (1997).
132. Morrison, S. J., Uchida, N. & Weissman, I. L. The biology of hematopoietic stem cells. *Annu. Rev. Cell Dev. Biol.* **11**, 35–71 (1995).
133. Visser, J. W., Bauman, J. G., Mulder, A. H., Eliason, J. F. & de Leeuw, A. M. Isolation of murine pluripotent hemopoietic stem cells. *J. Exp. Med.* **159**, 1576–1590 (1984).
134. Bunting, K. D. ABC Transporters as phenotypic markers and functional regulators of stem cells. *Stem Cells* **20**, 11–20 (2002).
135. Goodell, M. A., McKinney-Freeman, S. & Camargo, F. D. Isolation and characterization of side population cells. *Methods Mol. Biol.* **290**, 343–352 (2005).
136. Uchida, N., Dykstra, B., Lyons, K. J., Leung, F. Y. & Eaves, C. J. Different *in vivo* repopulating activities of purified hematopoietic stem cells before and after being stimulated to divide *in vitro* with the same kinetics. *Exp. Hematol.* **31**, 1338–1347 (2003).
137. Camargo, F. D., Chambers, S. M., Drew, E., McNagny, K. M. & Goodell, M. A. Hematopoietic stem cells do not engraft with absolute efficiencies. *Blood* **107**, 501–507 (2006).
138. Bradford, G. B., Williams, B., Rossi, R. & Bertoncello, I. Quiescence, cycling, and turnover in the primitive hematopoietic stem cell compartment. *Exp. Hematol.* **25**, 445–453 (1997).
139. Komori, T. *et al*. Targeted disruption of *Cbfa1* results in a complete lack of bone formation owing to maturational arrest of osteoblasts. *Cell* **89**, 755–764 (1997).
140. Otto, F. *et al*. Cbfa1, a candidate gene for cleidocranial dysplasia syndrome, is essential for osteoblast differentiation and bone development. *Cell* **89**, 765–771 (1997).
141. Adams, G. D. *et al*. Stem cell engraftment at the endosteal niche is specified by the calcium-sensing receptor. *Nature*. Dec 28, 2005 (doi:10.1038/nature04247 16382241).

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Competing interests statement
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Entrez Gene:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
CD34 | CD150 | CXCL12 | FLT3 | KIT | N-cadherin | SCA1 | SCF

FURTHER INFORMATION
Andreas Trumpp's homepage: http://www.isrec.ch/research/groups/research_groups_detail_eid_1668_lid_2.htm

SUPPLEMENTARY INFORMATION
See online article: S1 (table)
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