The haematopoietic stem cell niche at a glance

Cristina Lo Celso1,* and David T. Scadden2

1Imperial College London, Division of Cell and Molecular Biology, Sir Alexander Fleming building, South Kensington Campus, London SW7 2AZ, UK
2Center for Regenerative Medicine, Massachusetts General hospital, Boston, MA 02114 and Department of Stem cells and Regenerative Biology, Harvard University, Cambridge, MA 02138, USA

*Author for correspondence (clocelso@imperial.ac.uk)

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Haematopoietic stem cells (HSCs) regulate the balanced turnover of erythrocytes, platelets and all immune cells by switching between self-renewal, differentiation, quiescence and dormancy (Trumpp et al., 2010) and, thereby, maintain homeostasis both in the steady state as well as in response to injury. HSCs are among the longest studied and utilised somatic stem cells. However, they cannot yet be maintained and expanded in vitro because a complex and dynamic molecular crosstalk between HSC and their endogenous microenvironment (or ‘niche’) directs their fate. The importance of the stem cell niche in regulating HSC function was first postulated in 1978 by Ray Schofield, when observing that the spleen is unable to support HSCs in the same way that bone marrow can (Schofield, 1978). Since then, it has become clear that not only HSCs but all somatic stem cells maintain homeostasis because they sense and respond to the need of an organism for their differentiated progeny as well as to stem cells themselves. The stem cell niche is the functional and anatomical ‘node’ that allows integration of signals from the periphery into the appropriate stem cell behaviour. The constantly evolving technological and experimental approaches have provided insights into the nature of the HSC niche, the specific function of different niche components and how perturbations in this highly integrated system are involved in the development of haematopoietic disease.

In this Cell Science at a Glance article, we provide an update of the cellular and molecular components of the HSC niche, describe how they adapt in response to specific stimuli, and discuss their potential role in the development of haematopoietic disease. As several controversial observations have led to the most recently proposed niche models and because future studies are likely to further change our views again, we do not present a definitive, detailed portrait of the HSC niche but, instead, integrate currently accepted niche elements and dynamic models to provide an up-to-date overview. Box 1 contains details on the specific nomenclature used in this article. Box 2 contains a brief summary of recent developments.
Box 1. Notes on nomenclature
An increasing number of haematopoietic stem and progenitor cell (HSPC) subpopulations continues to be identified. Furthermore, the steady introduction of new markers has demonstrated the heterogeneity of cell populations used in previous studies. In this Cell Science at a Glance article, we use the term HSC when referring to the experimental demonstration of HSC function and the term HSPC to indicate phenotypically defined cell populations that have been shown to contain HSCs.

The term HSC niche often refers strictly to the microenvironment of the most primitive HSCs. However, as a result of the continuous experimental refinement of this cell population and the increasing – and often controversial – number of bone marrow components shown to affect HSC function, this term is best used to refer to the entire network of extrinsic factors that regulates HSC fate.

Finally, various osteoblastic subpopulations have been proposed to differentially interact with and affect HSCs, but a widespread consensus on markers that make their distinction possible is missing. We, therefore, use the terms osteoblast and osteoblastic cell to refer to the whole lineage and not to cells at a specific stage of differentiation or with a particular metabolic activity.

Experimental models and technological advances that were used to study the HSC niche.

Cellular components of the HSC niche
HSCs reside within the bone marrow, which presents a complex microenvironment that is made up of different cell types and extracellular elements. An increasing number of bone marrow lineages, structures and molecular components have been demonstrated to affect HSC fate and function.

The endosteal surface of the bone and cells of the osteoblastic lineage were shown first to be components of the HSC niche (Calvi et al., 2003; Grassinger et al., 2010; Lord et al., 1975; Visnjic et al., 2004; Zhang et al., 2003). Whether a specific subpopulation of osteoblastic cells is interacting with HSCs is currently under investigation. Recently, secreted phosphoprotein 1 [SPP1; also known as osteopontin (OPN) and one of the main extracellular proteins secreted by osteoblasts] and ALCAM (an adhesion molecule widely expressed across different lineages) were proposed as markers of a subpopulation of osteoblastic cells that affect HSC function. (Mayack and Wagers, 2008; Nakamura et al., 2010). Interestingly, haematopoiesis is dramatically affected by the conditional deletion of the ribonuclease Dicer in osteoprogenitors. However, the same deletion in fully mature, osteocalcin-positive osteoblasts does not lead to haematopoietic defects (Raaijmakers et al., 2010), and the selective ablation of terminally differentiated osteoblasts does not affect haematopoiesis either (Corral et al., 1998; Raaijmakers et al., 2010). The association between HSCs and osteoprogenitors might be rooted even earlier within the osteoblastic lineage, as was suggested by the fact that nestin-positive MSC-like cells are marrow stroma cells that can interact with HSCs (Mendez-Ferrer et al., 2010). Furthermore, human CD146+ osteoprogenitor cells are able to direct ectopic bone formation accompanied by haematopoietic seeding (Sacchetti et al., 2007). PDGFRα+ Sea1+ mesenchymal progenitors have been successfully transplanted and have been shown to localise to areas of the bone marrow that are generally recognised to contain HSC niches (Morikawa et al., 2009). Taken together, these studies show that osteoblastic cells at various stages of differentiation can support different HSC functions and states.

The endosteal HSC niche model was challenged, however, by immunofluorescence studies, which showed that the only bone marrow structure that is consistently located adjacent to HSCs is the sinusoidal vasculature (Kiel et al., 2005). Integrity and regeneration of bone marrow vasculature are, indeed, fundamental for HSC recovery from myeloablative injuries and following bone marrow transplantation (Hooper et al., 2009; Kobayashi et al., 2010). Several reports agree on the location of functional, engrafting haematopoietic stem and progenitor cells (HSPCs, Box 1) being near the endosteal surface but not exclusively adjacent to osteoblastic cells (Jiang et al., 2009; Lo Celso et al., 2009; Xie et al., 2009). Whereas endosteal surfaces are highly vascularised, the question remains whether HSCs that are located at varying distances from osteoblastic cells are functionally distinct from those located near osteoblastic cells.

Other HSC regulators include perivascular, non-endothelial supportive cells (Sugiyama et al., 2006), adipocytes, which have been shown to inhibit HSC engraftment (Naveiras et al., 2009), and the autonomous nervous system, which influences HSC mobilisation (Katayama et al., 2006). In addition, several cells of haematopoietic origin have a role in the HSC niche. For example, the activation of osteoclasts, a specialised subpopulation of endosteal macrophages that are responsible for bone resorption, leads to HSC egress from the bone marrow (Kollet et al., 2006), whereas their pharmacological inhibition leads to a reduction of HSC numbers (Lymperi et al., 2011). A different subpopulation of bone marrow macrophages, the ‘osteomacs’, form a canopy of cells near active osteoblasts and carry out the opposite role: their depletion leads to the loss of osteoblast activity and increased HSC mobilisation (Winkler et al., 2010). Very recently regulatory T cells have been demonstrated to make the HSC niche a site of immune privilege (Fujisaki et al., 2011).

The question remains whether we should think about one or several HSC niches. The observation that different HSPC localisations exist within the marrow and that a growing number of cell types are involved in HSC regulation (as illustrated above), together with the increasing number of reports that describe the heterogeneity of even highly purified HSPC populations (Dykstra et al., 2007; Lo Celso et al., 2009; Wilson et al., 2008), could be an indication of the complex microenvironments through which HSCs navigate. One proposed model suggests that osteoblastic cells provide a context for HSC dormancy, whereas a perivascular, quiescent niche provides an intermediate niche for activated HSCs that are ready to either generate differentiating progeny or revert to dormancy, depending on the needs of the organism (Malhotta and Kincade, 2009a; Trumpp et al., 2010). It is possible that the number of functional niches increases further if additional HSC subpopulations and states are identified. Real-time long-term cell tracking coupled with a greater number of reporter strategies to simultaneously highlight multiple stroma components and HSPC subpopulations will be instrumental to fully understand the interactions between HSCs and their niche.

Molecular regulators of HSC fate
Independent of the identity of the niche cell that generates a signal for the HSC, a multitude of molecular regulators of HSC fate have been described. Some are known products of osteoblastic cells; however, it is possible that an increasing number of these factors are produced by multiple cell types.

Secreted ligands and their receptors
The stroma-derived factor 1 (SDF1; also known as CXCL12)-C-X-C chemokine receptor type 4 (CXCR4) axis is the best-defined regulator of HSC localisation in the bone marrow. Osteoblastic cells produce SDF1 and upregulate its expression in response to irradiation (Xie et al., 2009). However, analysis of SDF1-reporter mice has highlighted numerous perivascular cells throughout the marrow that
are SDF1-positive (Sugiyama et al., 2006), which indicates that SDF1 directs HSC localisation not only near osteoblastic cells but probably also throughout perivascular areas.

If SDF1 and CXCR4 are responsible for HSC localisation, other cytokines, signalling pathways and adhesion molecules known to have a role in the HSC niche might, instead, regulate HSC fate. Indeed, the ligand–receptor pairs stem cell factor (SCF) and KIT, thrombopoietin (TPO) and myeloproliferative leukemia virus oncogene (MPL), as well as angiotension 1 (ANGPT1) and TIE2 tyrosine kinase (officially known as TEK) have pivotal roles in regulating the interaction between osteoblasts and HSCs and, specifically, HSC maintenance and in vivo quiescence (Arai et al., 2004; Barker, 1997; Yoshihara et al., 2007). Recently, angiotension-like 3 (ANGPTL3), which is primarily expressed by endothelial cells, has been shown to control HSC quiescence as well as the number of HSCs both in the steady state and following transplantation (Zheng et al., 2011).

**WNT signalling**

The WNT signalling cascade has been implicated with a role in HSC regulation; but this is still highly controversial, mostly because of the complexity of the signalling pathway itself (Malhotra and Kincade, 2009b). Following initial reports that the obliteration of the canonical WNT pathway does not affect HSCs, later studies that focussed on interactions between HSCs and their niche provided further detailed information on the involvement of this pathway (Cobas et al., 2004; Koch et al., 2008). It was shown that osteoblast-specific overexpression of the WNT inhibitor dickkopf homolog 1 (DKK1) leads to impairment of HSC self-renewal (Fleming et al., 2008). Similarly, the knockout of secreted frizzled-related protein 1 (SFRP1) – another negative modulator of WNT signalling – leads to an initial increase in long-term reconstituting HSPCs (LT-HSPCs), followed by their premature exhaustion depending on the size of the well (Kurth et al., 2009). In vivo, it could be shown that HSCs seeded on microwells actively produce their own ECM and undergo quiescence or proliferation depending on the chemical gradients (Haylock and Nilsson, 2006). Moreover, eicosanoids (including PGE2, as mentioned above) affect the strength of signalling cascades, and neurotransmitters regulate the response to ECM protein that was shown to influence HSC homeostasis (Renstrom et al., 2009). Lack of SPP1 leads to a stroma-dependent increase of LT-HSPCs and increased JAG1 and ANGPT1 expression in stroma cells, which perhaps explains how, in Spp1-deficient mice, HSC expansion is not accompanied by their exhaustion (Nilsson et al., 2005; Staal and Clevers, 2005).

Glycans are non-protein components of the bone marrow stroma that have a role in the HSC niche. They are likely to mediate the formation of chemokine and growth factor gradients (Haylock and Nilsson, 2006). Moreover, eicosanoids (including PGE2, as mentioned above) affect the strength of signalling cascades, and neurotransmitters regulate the response to ECM protein that was shown to influence HSC homeostasis (Renstrom et al., 2009). Lack of SPP1 leads to a stroma-dependent increase of LT-HSPCs and increased JAG1 and ANGPT1 expression in stroma cells, which perhaps explains how, in Spp1-deficient mice, HSC expansion is not accompanied by their exhaustion (Nilsson et al., 2005; Staal and Clevers, 2005).

For a number of years, the role of β1 integrins in the crosstalk between HSCs and their niche has been of interest. Integrin α4β1 (ITGA4; also known as VLA4) mediates HSC retention within the bone marrow microenvironment (Pries et al., 2006), whereas integrins α1β1 and α5β1 (ITGA3 and ITGA5; also known as VLA1 and VLA5, respectively) mediate adhesion of HSCs to – among others – SPP1 (Nilsson et al., 2005). Furthermore, these three integrins mediate SDF1 function (Peled et al., 2000). Interestingly, there is a link between WNT signalling and integrin expression in HSCs: the expression of constitutively active β-catenin leads to the loss of HSCs and rapid exhaustion of their progeny, but also to higher expression of integrin α2, β1 and β7 in HSPCs (Kirstetter et al., 2006).

Integrins interact with ECM proteins, but integrin α4β1 is also the main binding partner of vascular cell adhesion molecule 1 (VCAM1), which is expressed on the surface of endothelial cells (Ulyanova et al., 2007) as well as cells of the osteoblastic lineage (Jiang et al., 2009). Interestingly, VCAM1 expression correlates with HSC homing (Lewandowski et al., 2010) and is upregulated in response to WNT signalling (Malhotra and Kincade, 2009a). Cadherins, which make up another class of adhesion molecules, might also contribute to HSC fate but their role in HSC homeostasis remains controversial (Levesque et al., 2010). Future studies will indicate whether the HSC niche is similar to other niches in that the direct interaction of HSCs with osteoblastic, endothelial and other cell types is fundamental to ensure their correct function.

**Chemical gradients**

Calcium and oxygen are the main chemical elements that have been studied in relation to the HSC niche. Ca2+ is released by osteoclasts during bone resorption, which leads to the formation of a concentration gradient that...
spreads out from the endosteal surface (Levesque et al., 2010). HSCs express the G-protein coupled Ca${^{2+}}$-sensing receptor (CASR) and depend on it for their peri-endosteal localisation and function (Adams et al., 2006).

In contrast to the acknowledged role of Ca${^{2+}}$ in the HSC niche, the relevance of oxygen tension is a controversial topic. Adaptations of the protocol for the isolation of side populations (Goodell, 2005), which use Hoechst dye in vivo staining to label efficiently perfused cells, suggested that HSCs reside in hypoxic areas of the bone marrow (Parmar et al., 2007; Winkler et al., 2010). However, both endosteal and non-endosteal LT-HSPCs are localised near vasculature (Kiel et al., 2005; Lo Celso et al., 2009). Therefore, in vivo measurements of bone marrow O$_2$ concentration are necessary to definitively assess whether O$_2$ gradients are present within the marrow space and what their defining features and function are. This question has recently gained further attention, as O$_2$ levels were shown to affect transcription and WNT signalling through the activity of HIF1α, the hypoxia-responsive transcription factor that is also expressed in HSPCs (Levesque et al., 2007).

The HSC niche is a paramount example of the complex molecular interactions that take place in living tissues, and the evolutionary robustness of vertebrate haematopoietic systems undoubtedly relies on the intricacy of its molecular regulation. Increasingly detailed spatio-temporal analysis of the molecular composition of the HSC niche will allow us to fit current and new data into a functional HSC niche model.

**HSC niche dynamics**

All somatic stem cell niches are characterised by intrinsic dynamics, due to the oscillation of stem cells between quiescence, self-renewal and differentiation. The HSC niche, however, has to accommodate a unique trait: HSCs periodically leave the niche and enter circulation (‘mobilisation’) to later re-enter the bone marrow space (‘homing’) (Bhattacharya et al., 2009; Wright et al., 2001). This physiological phenomenon is exploited therapeutically during bone marrow transplantation. Because bone marrow engraftment requires conditioning of the recipient by means of irradiation or chemotherapy, bone marrow transplantation provides a prime example of the remarkable regenerative potential of the HSC niche. The dramatic perturbations caused by pharmacological HSC mobilisation and transplantation have been studied for a long time in order to improve clinical practice, and recent technological advances have allowed to unravel the more subtle changes that occur in the HSC niche during physiological responses and throughout the development of disease.

**Induced HSC mobilisation**

Granulocyte colony-stimulating factor [G-CSF, officially known as colony stimulating factor 3 (CSF3)] is the most widely used mobilising agent in clinical practice. It has a highly multifaceted mode of action: G-CSF administration results in the transient reduction of SDF1 expression, the activation of metalloproteases that cleave SCF, VCAM1 and SDF1, as well as the release of norepinephrine from the sympathetic nervous system, which affects the morphology of osteoblasts and their ability to retain HSCs within the niche (Brouard et al., 2010; Katayama et al., 2006). Endocannabinoids and reactive oxygen species (ROS) have recently been identified as non-protein mediators of the effects of G-CSF action (Jiang et al., 2011; Jiang et al., 2010; Tesio et al., 2011). Interestingly, whereas HSC mobilisation is normally transient, the continuous administration of the SDF1 antagonist AMD3100 can lengthen this process (Bonig et al., 2009).

**HSC transplantation**

HSC homing is the first step of a successful transplantation and crucially depends on the SDF1–CXCR4 signalling axis and β1 integrins. Subsequently, the CASR, in response to a Ca$^{2+}$ gradient, mediates the lodging of HSC near the endosteum (see above) (Adams et al., 2006; Jung et al., 2006). Although HSCs exit and re-enter the niche normally, some molecules are dispensable during steady-state haematopoiesis but are crucial for transplanted HSC homing. For example, the stimulatory class of the Gα subunit of G-protein-coupled receptors (Gαs) is necessary for transplanted HSPCs to extravasate and reach the bone marrow space (Adams et al., 2009). Furthermore, CD34 might have a role in HSC movement and transmigration to the niche but, so far, CD34-dependent defects have only been observed in very specific transplantation settings (Nielsen and McNagny, 2007).

Bone marrow conditioning with cytotoxic agents or irradiation prior to transplantation triggers several molecular signals and is crucial for HSC homing in the endosteal region (Jiang et al., 2009; Lo Celso et al., 2009). The consequences of conditioning on cells of the osteoblastic and other stroma lineages are only partially known, but upregulation of SDF1, ROS-mediated induction of VCAM1 and vascular endothelial growth factor (VEGF)-mediated endothelial recovery have been highlighted as part of the regenerative process triggered by lethal irradiation (Hooper et al., 2009; Lewandowski et al., 2010; Xie et al., 2009).
Physiological HSC trafficking

Recent studies uncovered the ability of the HSC niche to orchestrate dynamic responses to sub-lethal injuries through the recruitment of dormant HSCs. An analysis of the response to interferon α (IFNα) treatment (which mimics activation of the immune response) was the first study that highlighted a clear function for Scf1 (stem cell antigen 1) (Essers et al., 2009), which has long been recognised as a marker of mouse HSPCs.

The physiological egress of HSCs from, and their return to, the bone marrow niche is probably regulated by mechanisms that are similar to those linked to successful mobilisation, engraftment and recovery from injury, albeit on a smaller scale. Circadian rhythms affect niche anatomy and the number of circulating HSCs (Mendez-Ferrer et al., 2008), and bone remodelling itself might have a crucial role in HSC homeostasis (Levesque et al., 2010). Ageing is another physiological process that is likely to affect HSC–niche interactions. Although long-term follow-up of endogenous HSCs is currently unachievable, differences in cell volume oscillation and position relative to the endosteal surface were observed in young and old transplanted HSPCs (Kohler et al., 2009).

The HSC niche in disease

There is evidence for the HSC niche both supporting the development of and being affected by haematopoietic disease. Aberrant bone marrow stroma can lead not only to loss of HSC function but also to myelofibrosis and myelodysplastic syndromes (Lane et al., 2009; Raaijmakers et al., 2010; Walkley et al., 2007a). For example, mice with defective microRNA processing in the osteoblastic lineage develop profound bone defects as well as severe myelodysplasia, which can occasionally develop into leukaemia (Raaijmakers et al., 2010). Whereas it is clear that aggressive leukaemia depends on the accumulation of genetic abnormalities in cells of the haematopoietic lineage, this model indicates that the bone marrow stroma has a role in disease development, presumably by exerting different selection criteria that support leukaemia cells or confer a disadvantage to normal haematopoietic cells. Niche aberrations might, therefore, favour the development of disease. In addition, leukaemia cells might acquire the ability to respond differently to physiological niche signals than regular HSCs. For example, transforming growth factor β (TGFβ) is thought to contribute to the dormancy of normal HSCs (Yamazaki et al., 2009) and, by contrast, contributes to the survival and proliferation of leukaemia-initiating cells in a model of chronic myelogenous leukaemia (Naka et al., 2010).

Whether normal HSCs and leukaemia stem cells compete for the same niche space is still an open question, but it appears to be that normal haematopoiesis is impaired in the setting of leukaemia. Indeed, the homing pattern of human HSPCs is dramatically altered when they are transplanted into recipient leukaemic mice (Colmone et al., 2008). Whether leukaemia impairs HSC niche function is unclear (Podor et al., 2009), but human chronic myeloid leukaemia stem (or leukaemia-initiating) cells have been shown to reside near the endosteal surface when injected into newborn irradiated recipient mice (Saito et al., 2010). HSCs, whether normal or leukaemic, might have a role in shaping their niche and it remains an interesting question, whether that interaction can be pharmacologically manipulated to selectively affect the support of normal or malignant cells (Colmone et al., 2008).

Perspectives

The number of known cellular components and molecular mechanisms that regulate HSC–niche interactions has dramatically increased during the past few years. An interesting trend in the HSC niche field has been the progression from several controversies to the clarification that HSCs present a scenario that is more complex than previously thought. Whereas initial studies highlighted the role of osteoblastic cells in regulating HSC number and function, it is now widely accepted that several other stromal cell types, ranging from mesenchymal stem cells to various differentiated lineages, interact with HSC and influence their fate through an array of secreted and cell–cell adhesion molecules as well as by affecting the protein and chemical composition of bone marrow extracellular space. The current picture of the components involved in the HSC niche is likely to grow in complexity, and the same is true for the intricacy of signalling pathways and subtleness of effects based on intensity and localisation of each signal.

Further technological advances and increasingly refined experimental models will allow us to explore new dimensions of the HSC niche itself and to unravel several points that are currently under discussion. For example, an open question is how many functionally distinct HSC niches exist and whether they are spatially segregated or, rather, represent dynamic oscillations in the bone marrow microenvironment. Studies on invertebrate niches have already indicated their ability to tightly regulate symmetry during stem cell division and to instruct the reversion of differentiating cells to a stem cell stage. Further development of imaging-based and functional assays will allow the investigation of whether this is also the case for the HSC niche. Finally, understanding the relationship between normal and diseased HSC–niche interactions will allow us to harness these mechanisms for therapeutic purposes through effective ex vivo expansion of HSCs and the development of new treatments for haematopoietic disease.

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