Special Issue: Hematopoietic and Mesenchymal Stem Cells

Mini Review

# Hematopoietic stem cells and niche cell populations

**Fumio Arai**

Department of Cell Differentiation, The Sakaguchi Laboratory of Developmental Biology, School of Medicine, Keio University, Tokyo, Japan

**The interactions of stem cells with their supportive microenvironmental niches are mediated by signaling networks that control the balance between cellular self-renewal and differentiation. In hematopoietic stem cells (HSCs), the bone marrow (BM) supports both of these processes within specialized niches, namely, osteoblastic and perivascular niche, which contain supportive cellular and non-cellular elements. This review discusses HSC niches and niche cell populations, focusing on the osteoblastic niche cells in three fractions: osteoblast-enriched ALCAM$^{+}$Sca-1$^{-}$ and ALCAM$^{-}$Sca-1$^{-}$, and immature mesenchymal cell-enriched ALCAM$^{-}$Sca-1$^{+}$ cells. Gene expression profiling showed that the ALCAM$^{-}$Sca-1$^{+}$ fraction highly expressed cytokine-related genes whereas in the ALCAM$^{+}$Sca-1$^{-}$ fraction the predominantly expressed genes were those related to cell adhesion. In addition, by using single-cell gene expression analysis, we identified an osteoblastic marker$^{low/-}$ subpopulation in ALCAM$^{+}$Sca-1$^{-}$ cells, which includes cells that express relatively high levels of pluripotent markers. Together, these findings indicate that multiple cell populations cooperatively support HSCs in the osteoblastic niche. Understanding the niche signals that regulate HSC maintenance and terminal differentiation could provide the basis for niche-based therapies that protect HSCs from various stresses and promote the *ex vivo* expansion of HSCs.**

**Rec.5/30/2012, Acc.7/9/2012, pp152-157**

Correspondence should be addressed to:
Fumio Arai, D.D.S., Ph. D., Department of Cell Differentiation, The Sakaguchi Laboratory of Developmental Biology, School of Medicine, Keio University, 35 Shinano-machi, Shinjuku-ku, Tokyo 160-8582, Japan. Phone: +81-3-5363-3475, Fax: +81-3-5363-3474, E-mail: farai@a3.keio.jp

**Key words** stem cell niche, mesenchymal stem/progenitor cells, osteoblast, hematopoietic stem cell

Stem cells are characterized by their self-renewal capacity as well as their potential to differentiate into single or multiple types of daughter cells. They are maintained in different tissues by general genetic programs[1, 2], although the critical genes responsible for the functioning of these programs are likely to differ across stem cell types.

Hematopoietic stem cells (HSCs) are responsible for blood cell production throughout the lifetime of the indi-

vidual. Among bone marrow (BM) HSCs, which are the best-characterized stem cells, various subsets can be isolated using cell surface markers[3]. HSCs differentiate into several blood cell lineages[4-6].

Stem cells reside within a supportive microenvironmental niche composed of cellular and non-cellular components. The interactions between HSCs and niche supportive cells are mediated by signaling networks that control the balance between stem cell self-renewal and differentiation[7-9]. Furthermore, the balance between HSC quiescence and activation (proliferation and migration) requires the cooperative regulation exerted by the non-cellular elements: cytokines, chemokines, adhesion molecules, proteolytic enzymes, neurotransmitters and transcription factors[8, 10]. An understanding of the influences controlling the fate of BM-HSCs therefore requires elucidation of the molecular interactions between these cells and their niches.

## The hematopoietic niche

In hematopoiesis in the adult, the BM supports both the self-renewal and the differentiation of HSCs at particular sites. The localization of HSCs in the endosteum (the border between the bone and the BM) and in perivascular sites of the BM led to the identification of osteoblastic (also called endosteal) and perivascular niches, both of which are broadly distributed in the BM. Thus, within the BM HSC niche, HSCs interact with both of the aforementioned niches (Fig.1). Indeed, the vascular network extends into the outermost regions of the endosteal surfaces, suggesting a close relationship between HSCs, the osteoblastic niche, and the perivascular niche in the regulation of hematopoiesis, bone formation, and vascular remodeling[11, 12].

## Niche cell populations supporting hematopoietic stem cells

The regulation of HSC maintenance in the hematopoietic niche requires numerous cell types: mesenchymal stem/progenitor cells (MSCs/MPCs), osteoblasts, reticular cells (Cxcl12 abundant reticular cells: CAR cells), endothelial cells, perivascular cells, adipocytes, osteoclasts, macrophages, regulatory T cells, cells of the sympathetic nervous system, and Schwann cells[13-20].

In particular, the Nestin$^+$ MSC population was shown to be distributed within the perivascular area in close association with HSCs and catecholaminergic nerve fibers. Compared with their Nestin$^-$ counterparts, Nestin$^+$ MSCs

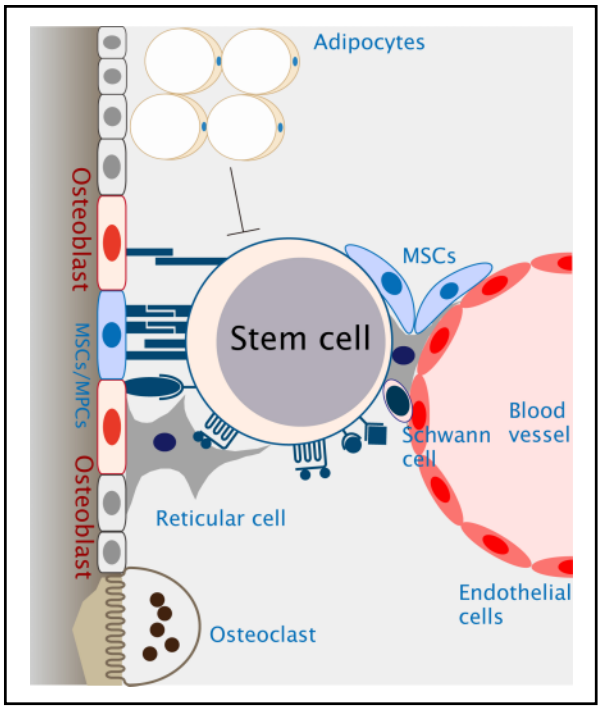


Fig.1 The HSC niche
HSCs interact with both osteoblastic and perivascular niches in the BM. Many types of cells, including mesenchymal stem/progenitor cells (MSCs/MPCs), Schwann cells, endothelial cells, reticular cells, osteoblasts, adipocytes, and osteoclasts, contribute to the regulation of HSCs.

highly express genes related to HSC regulation, such as *Cxcl12, Kitl,* and *Angpiopoietin1* (*Angpt1*). Furthermore, the depletion of Nestin$^+$ MSCs was shown to cause a decline of HSCs in the BM[19], suggesting that Nestin$^+$ MSCs are a central cellular component of the HSC niche in the BM[19].

We previously reported that Angpt1 and Mpl/Thrombopoietin (Thpo) signaling between HSCs and osteoblastic niche cells is critical for the enhancement of cell-to-cell and cell-to-extracellular matrix interactions of HSCs with niche cells, as well as for the maintenance the cell cycle quiescence of HSCs in the endosteal area[21, 22]. However, the cells in the endosteal region are a heterogeneous population in terms of their differentiation status and accompanying functions[23, 24], and their precise cellular and molecular contributions to the HSC-supportive microenvironment is unclear.

In a series of experiments we isolated endosteal niche cells, with the aim of characterizing their function with respect to the maintenance of HSCs. Subpopulations of the endosteal cell fraction were obtained from the mouse BM

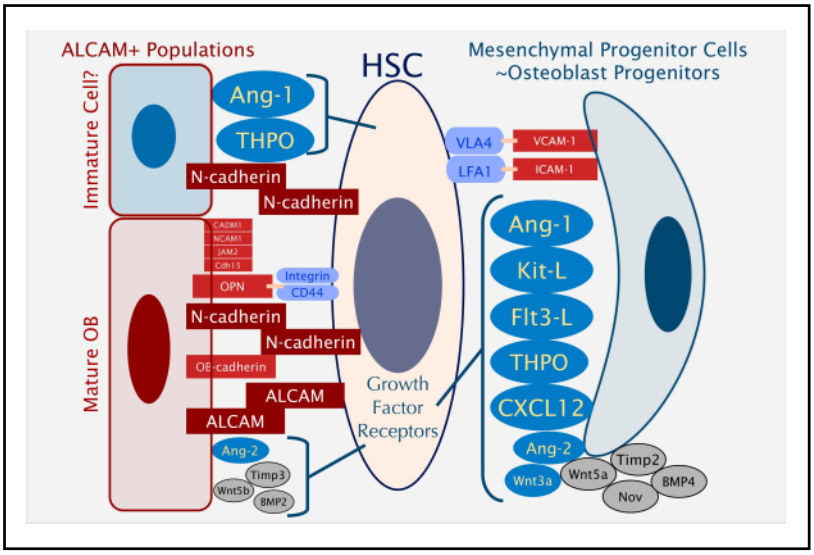


Fig.2 Role of endosteal cell populations in HSC regulation

In the osteoblastic niche, mesenchymal progenitor cells, osteoprogenitor cells, and osteoblasts cooperatively regulate HSCs. Mesenchymal progenitor cells and osteoprogenitor cells produce cytokines that control HSC proliferation and quiescence. By contrast, mature osteoblastic cells express multiple adhesion molecules, such as N-cadhein, OB-cadherin, and ALCAM, and therefore may serve as a scaffold for HSC anchoring, in addition to physically regulating HSCs via cell-to-cell adhesion. The osteoblastic marker$^{low/-}$ sub-population of ALCAM$^{+}$Sca-1$^{-}$ cells express pluripotent stem cell markers and produce cytokine and cell adhesion molecules. The potential of these cells in terms of their self-renewal activity, differentiation potential, and function in HSC maintenance remains to be elucidated.

based on their expression of the markers ALCAM and Sca-1. Bone-associated cells were isolated by collagenase treatment of tibial and femoral bone fragments. Non-hematopoietic and non-endothelial cells in the CD45$^{-}$CD31$^{-}$Ter119$^{-}$ fraction were enriched and further subdivided into three fractions: ALCAM$^{+}$Sca-1$^{-}$, ALCAM$^{-}$Sca-1$^{+}$, and ALCAM$^{-}$Sca-1$^{-}$ cells[25]. Analysis of the differentiation potential of these fractions together with conventional gene-expression PCR revealed that ALCAM$^{+}$Sca-1$^{-}$, ALCAM$^{-}$Sca-1$^{-}$, and ALCAM$^{-}$Sca-1$^{+}$ cells are mature osteoblasts, immature osteoblasts, and mesenchymal progenitor cells, respectively. Cells of the ALCAM$^{-}$Sca-1$^{+}$ population expressed the highest levels of MSC-associated genes, such as Endoglin and CD90, and differentiated into osteoblasts and adipocytes, By contrast, their TGF$\beta$3-iduced differentiation into chondrocytes was significantly lower than that of previously-reported MSC population[26, 27]. It was therefore concluded that bone-associated ALCAM$^{-}$Sca-1$^{+}$ cells are progeny of Nestin$^{+}$ MSCs.

## Function of endosteal niche cell populations in the maintenance of HSCs

In examining the ability of the three endosteal cell populations to maintain HSC activity, we found that all of them supported the long-term repopulation activity of HSCs. In particular, ALCAM$^{+}$Sca-1$^{-}$ cells showed robust supporting activity for HSCs when placed in an *in vitro* coculture. LSK cells expressed significantly higher levels of homing- and cell-adhesion-related genes, such as *Cxcr4, Itga2b, Itgb2, Cd44, Cdh2* (*N-cadherin*), and *Vcam1*, when cocultured with ALCAM$^{+}$Sca-1$^{-}$ than when cocultured with ALCAM$^{-}$Sca-1$^{+}$ cells. In addition, ALCAM$^{+}$Sca-1$^{-}$ cells significantly up-regulated the expression of *Gfi1, Hoxb4, Cdkn1c, Foxo3,* and *Sox2* in LSK cells. These data suggested that, during culture, ALCAM$^{+}$Sca-1$^{-}$ cells either enhance the long-term repopulation (LTR) activity of HSCs or enrich a cell population with higher intrinsic LTR activity.

Microarray analysis revealed that cytokine- and cell adhesion-related genes are expressed in distinct endosteal cell fractions. Specifically, cytokine-related genes are highly expressed in ALCAM$^{-}$Sca-1$^{+}$ cells, suggesting that this population regulates HSCs through the production of cytokines influencing both HSC proliferation and quiescence. By contrast, ALCAM$^{+}$Sca-1$^{-}$ cells expressed genes for multiple cell adhesion molecules, indicating that this sub-population physically regulates HSC quiescence via cell adhesion molecules. We therefore hypothesized that multiple endosteal populations cooperatively regulate HSC function in the BM osteoblastic niche (Fig.2).

## Developmental changes in endosteal cell gene expression

We also investigated changes in the features of ALCAM$^{+}$Sca-1$^{-}$, ALCAM$^{-}$Sca-1$^{-}$, and ALCAM$^{-}$Sca-1$^{+}$ cells during postnatal development of the BM. Since postnatally, HSCs undergo a shift from a cycling to a quiescent

state[21], the function of the BM niche likewise changes, from supporting expansion to the maintenance of HSC quiescence. Consistent with this scenario, osteoblast populations isolated from the bones of younger mice (2-4 weeks old) expressed high levels of genes encoding VEGFa, Angpt2, and MMPs. MMP9 is known to induce the shedding of membrane-anchored growth factors such as Kitl[28, 29], while soluble-Kitl reportedly induces HSC proliferation. We therefore hypothesized that endosteal cells induce HSC proliferation through the activation of vascular remodeling and the production of soluble growth factors during postnatal development.

## Identification of immature cell populations in endosteal cells

Recently, there have been tremendous advances in the techniques for analyzing gene expression patterns at the single-cell level. Unlike conventional gene expression analyses using pooled cell samples, single-cell analysis can be used to identify specific sub-populations within heterogeneous cell populations. Thus, using single-cell real time PCR array analysis (Dynamic Array™, Fluidigm), we characterized ALCAM$^+$Sca-1$^-$ and ALCAM$^-$Sca-1$^+$ cell fractions in greater detail, analyzing the expression of genes encoding osteoblastic markers, cytokine signals, extracellular matrices, cell adhesion molecules, MMPs, MSC markers, and pluripotent stem cell markers in single-cell samples of the two fractions. The general trend in the gene expression patterns of each fraction was consistent with the results of both conventional real time PCR and microarray analysis of pooled cell samples. Interestingly, the ALCAM$^+$Sca-1$^-$ fraction was determined to be heterogeneous, such that a unique subpopulation (osteoblastic marker$^{low/-}$, comprising ~36% of ALCAM$^+$Sca-1$^-$ cells) was identified in which osteoblastic markers were expressed at very low levels or not at all. Furthermore, approximately 40% of these osteoblastic marker$^{low/-}$ ALCAM$^+$Sca-1$^-$ cells expressed genes encoding BM-HSC niche-related cytokines, such as *Angtp1* and *Thpo*. Also of interest was the observation that ~ 30% of the osteoblastic marker$^{low/-}$ cells expressed pluripotent stem cell markers, such as *Sox2, Oct3/4,* and *Nanog,* at relatively high levels compared with ALCAM$^+$Sca-1$^-$ and ALCAM$^-$Sca-1$^+$ cells (Fig.2). These data indicated that the endosteal area contains cells expressing these markers.

## Conclusion and future directions

Recent progress in the research of stem cell niches has advanced our understanding of the cellular and molecular constituents of the HSC niche. It is now clear that this niche is multicellular, with multiple stromal cell types contributing to HSC regulation. Understanding the niche signals that regulate HSC maintenance and terminal differentiation could provide the basis for niche-based therapies targeting both the protection of HSCs from various stresses and the *ex vivo* expansion of HSCs.

The potential of the osteoblastic marker$^{low/-}$ ALCAM$^+$Sca-1$^-$ cells, in terms of their self-renewal activity, differentiation potential, and function in HSC maintenance, remains to be determined but this potential certainly constitutes an area of clinical and therapeutic interest. In addition, by applying more detailed fractionation methods based on single-cell gene expression, a detailed characterization of niche cell components may soon be possible.

### Acknowledgments

I appreciate all members of Department of Cell Differentiation, School of Medicine, Keio University for insightful comments in the preparation of the manuscript.

### Sources of Funding

This work was supported by the Funding Program for Next Generation World-Leading Researchers (NEXT Program).

### Conflict of interests

None

### References

1) Ramalho-Santos M, Yoon S, Matsuzaki Y, Mulligan RC, Melton DA: "Stemness": transcriptional profiling of embryonic and adult stem cells. Science. 2002; 298: 597-600.
2) Ivanova NB, Dimos JT, Schaniel C, Hackney JA, Moore KA, Lemischka IR: A stem cell molecular signature. Science. 2002; 298: 601-604.
3) Wilson A, Laurenti E, Oser G, van der Wath RC, Blanco-Bose W, Jaworski M, Offner S, Dunant CF, Eshkind L, Bockamp E, Lio P, Macdonald HR, Trumpp A: Hematopoietic stem cells reversibly switch from dormancy to self-renewal during homeostasis and repair. Cell. 2008; 135: 1118-1129.
4) Akashi K, Traver D, Miyamoto T, Weissman IL: A clonogenic common myeloid progenitor that gives rise

to all myeloid lineages. Nature. 2000; 404: 193-197.
5) Kondo M, Weissman IL, Akashi K: Identification of clonogenic common lymphoid progenitors in mouse bone marrow. Cell. 1997; 91: 661-672.
6) Adolfsson J, Mansson R, Buza-Vidas N, Hultquist A, Liuba K, Jensen CT, Bryder D, Yang L, Borge OJ, Thoren LA, Anderson K, Sitnicka E, Sasaki Y, Sigvardsson M, Jacobsen SE: Identification of Flt3+ lympho-myeloid stem cells lacking erythro-megakaryocytic potential a revised road map for adult blood lineage commitment. Cell. 2005; 121: 295-306.
7) Moore KA, Lemischka IR: Stem cells and their niches. Science. 2006; 311: 1880-1885.
8) Wilson A, Trumpp A: Bone-marrow haematopoietic-stem-cell niches. Nat Rev Immunol. 2006; 6: 93-106.
9) Morrison SJ, Spradling AC: Stem cells and niches: mechanisms that promote stem cell maintenance throughout life. Cell. 2008; 132: 598-611.
10) Lapidot T, Dar A, Kollet O: How do stem cells find their way home? Blood. 2005; 106: 1901-1910.
11) Lo Celso C, Fleming HE, Wu JW, Zhao CX, Miake-Lye S, Fujisaki J, Cote D, Rowe DW, Lin CP, Scadden DT: Live-animal tracking of individual haematopoietic stem/progenitor cells in their niche. Nature. 2009; 457: 92-96.
12) Xie Y, Yin T, Wiegraebe W, He XC, Miller D, Stark D, Perko K, Alexander R, Schwartz J, Grindley JC, Park J, Haug JS, Wunderlich JP, Li H, Zhang S, Johnson T, Feldman RA, Li L: Detection of functional haematopoietic stem cell niche using real-time imaging. Nature. 2009; 457: 97-101.
13) Naveiras O, Nardi V, Wenzel PL, Hauschka PV, Fahey F, Daley GQ: Bone-marrow adipocytes as negative regulators of the haematopoietic microenvironment. Nature. 2009; 460: 259-263.
14) Sacchetti B, Funari A, Michienzi S, Di Cesare S, Piersanti S, Saggio I, Tagliafico E, Ferrari S, Robey PG, Riminucci M, Bianco P: Self-renewing osteoprogenitors in bone marrow sinusoids can organize a hematopoietic microenvironment. Cell. 2007; 131: 324-336.
15) Sugiyama T, Kohara H, Noda M, Nagasawa T: Maintenance of the hematopoietic stem cell pool by CXCL12-CXCR4 chemokine signaling in bone marrow stromal cell niches. Immunity. 2006; 25: 977-988.
16) Fujisaki J, Wu J, Carlson AL, Silberstein L, Putheti P, Larocca R, Gao W, Saito TI, Lo Celso C, Tsuyuzaki H, Sato T, Cote D, Sykes M, Strom TB, Scadden DT, Lin CP: In vivo imaging of Treg cells providing immune privilege to the haematopoietic stem-cell niche. Nature. 2011; 474: 216-219.
17) Yamazaki S, Ema H, Karlsson G, Yamaguchi T, Miyoshi H, Shioda S, Taketo MM, Karlsson S, Iwama A, Nakauchi H: Nonmyelinating Schwann cells maintain hematopoietic stem cell hibernation in the bone marrow niche. Cell. 2011; 147: 1146-1158.
18) Katayama Y, Battista M, Kao WM, Hidalgo A, Peired AJ, Thomas SA, Frenette PS: Signals from the sympathetic nervous system regulate hematopoietic stem cell egress from bone marrow. Cell. 2006; 124: 407-421.
19) Mendez-Ferrer S, Michurina TV, Ferraro F, Mazloom AR, Macarthur BD, Lira SA, Scadden DT, Ma'ayan A, Enikolopov GN, Frenette PS: Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. Nature. 2010; 466: 829-834.
20) Chow A, Lucas D, Hidalgo A, Mendez-Ferrer S, Hashimoto D, Scheiermann C, Battista M, Leboeuf M, Prophete C, van Rooijen N, Tanaka M, Merad M, Frenette PS: Bone marrow CD169+ macrophages promote the retention of hematopoietic stem and progenitor cells in the mesenchymal stem cell niche. J Exp Med. 2011; 208: 261-271.
21) Arai F, Hirao A, Ohmura M, Sato H, Matsuoka S, Takubo K, Ito K, Koh GY, Suda T: Tie2/angiopoietin-1 signaling regulates hematopoietic stem cell quiescence in the bone marrow niche. Cell. 2004; 118: 149-161.
22) Yoshihara H, Arai F, Hosokawa K, Hagiwara T, Takubo K, Nakamura Y, Gomei Y, Iwasaki H, Matsuoka S, Miyamoto K, Miyazaki H, Takahashi T, Suda T: Thrombopoietin/MPL signaling regulates hematopoietic stem cell quiescence and interaction with the osteoblastic niche. Cell Stem Cell. 2007; 1: 685-697.
23) Yin T, Li L: The stem cell niches in bone. J Clin Invest. 2006; 116: 1195-1201.
24) Kiel MJ, Morrison SJ: Uncertainty in the niches that maintain haematopoietic stem cells. Nat Rev Immunol. 2008; 8: 290-301.
25) Nakamura Y, Arai F, Iwasaki H, Hosokawa K, Kobayashi I, Gomei Y, Matsumoto Y, Yoshihara H, Suda T: Isolation and characterization of endosteal niche cell populations that regulate hematopoietic stem

cells. Blood. 2010; 116: 1422-1432.
26) Morikawa S, Mabuchi Y, Kubota Y, Nagai Y, Niibe K, Hiratsu E, Suzuki S, Miyauchi-Hara C, Nagoshi N, Sunabori T, Shimmura S, Miyawaki A, Nakagawa T, Suda T, Okano H, Matsuzaki Y: Prospective identification, isolation, and systemic transplantation of multipotent mesenchymal stem cells in murine bone marrow. J Exp Med. 2009; 206: 2483-2496.
27) Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR: Multilineage potential of adult human mesenchymal stem cells. Science. 1999; 284: 143-147.
28) Gallea-Robache S, Morand V, Millet S, Bruneau JM, Bhatnagar N, Chouaib S, Roman-Roman S: A metalloproteinase inhibitor blocks the shedding of soluble cytokine receptors and processing of transmembrane cytokine precursors in human monocytic cells. Cytokine. 1997; 9: 340-346.
29) Heissig B, Hattori K, Dias S, Friedrich M, Ferris B, Hackett NR, Crystal RG, Besmer P, Lyden D, Moore MA, Werb Z, Rafii S: Recruitment of stem and progenitor cells from the bone marrow niche requires MMP-9 mediated release of kit-ligand. Cell. 2002; 109: 625-637.