

## COMMENTARIES

### Clarifying The Contributions of Distinct Mesenchymal Populations in Supporting Hematopoiesis

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**Commentary on:** 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 May 14. [Epub ahead of print]

**Osteoblasts are now a well-recognized component of the hematopoietic stem cell (HSC) niche, and numerous pathways that mediate cross-talk between osteoblasts and HSCs have been identified. The cells of the osteoblast lineage are a heterogeneous population, and growing evidence suggests that populations at differing stages of osteogenic differentiation may play distinct roles in supporting hematopoietic development. Nakamura *et al.* have fractionated non-hematopoietic, non-endothelial cells of the bone marrow based on expression of ALCAM and Sca-1, and find that subpopulations have differing abilities to support long-term reconstitution (LTR) activity of HSCs (1). In particular, the ALCAM<sup>+</sup>Sca-1<sup>-</sup> population, containing differentiated osteoblasts, provides the greatest support for HSCs and expresses higher levels of cell adhesion molecules. These findings represent an important advance in understanding the relative contributions of different subsets of the osteoblast lineage to the bone marrow hematopoietic microenvironment.**

Since Schofield first proposed that the bone marrow microenvironment serves a crucial function in support of HSCs, the cellular identity and function of such stromal cells has been of great interest (2). In 1994, Taichman and Emerson demonstrated that osteoblasts could support hematopoiesis *in vitro* (3). While mature osteoblasts line the bone (endosteal) surface and secrete extracellular matrix, osteoblast progenitors

derived from mesenchymal stem cells are likely located in the bone marrow and comprise one subset of stromal cells. However, identification of these progenitors *in vivo* has been limited by the paucity of cell surface markers. Anatomic evidence has long suggested that HSCs are located in close proximity to mature osteoblasts along the endosteal surface, and in recent years genetic manipulation of osteoblast number has confirmed their crucial role in the HSC niche (4-6). However, earlier precursors in the osteoblast lineage may provide support for distinct subsets of hematopoietic cells. In particular, several groups have found that osteoblastic cells can also influence the differentiation of B cell precursors (7-9), and studies in mice have implicated the involvement of osteoblast progenitors (8). Therefore, in order to more completely understand the molecular mechanisms underlying crosstalk between the skeletal and hematopoietic systems, the ability to purify or enrich specific subpopulations of both osteoblast and hematopoietic lineages will be paramount.

In recent years, progress has been made in the area of isolating mesenchymal progenitors for the characterization of hematopoietic-supporting potential. Chan *et al.* demonstrated that CD105<sup>+</sup>Thy1<sup>-</sup> cells from fetal endochondral bones can give rise to ectopic bone and marrow cavity with hematopoietic reconstitution, while CD105<sup>+</sup>Thy1<sup>+</sup> progenitors, which express higher levels of the mature osteoblast marker osteocalcin, can form only bone via intramembranous ossification (10). Mayack

*et al.* have shown that cells isolated on the basis of expression of the osteoblast marker osteopontin can regulate HSC proliferation and mobilization (11).

Nakamura and colleagues now report the use of ALCAM, a cell-adhesion molecule, and Sca-1, a marker of progenitor cells, to fractionate bone marrow stromal cells into distinct ALCAM<sup>+</sup>Sca-1<sup>-</sup>, ALCAM<sup>-</sup>Sca-1<sup>-</sup>, and ALCAM<sup>-</sup>Sca-1<sup>+</sup> populations (1). Although all three fractions have osteogenic potential, alkaline phosphatase expression is detectable only in freshly isolated Sca-1<sup>-</sup> cells, suggesting that osteoblasts are enriched in this subset. Within the Sca-1<sup>-</sup> population, expression of osteocalcin is highest in the ALCAM<sup>+</sup> cells. Furthermore, although all three groups can maintain LTR activity of co-cultured HSCs, ALCAM<sup>+</sup>Sca-1<sup>-</sup> cells significantly enhance LTR activity. Microarray studies reveal increased expression of cell-adhesion markers and osteoblast markers within this fraction of mesenchymal cells.

In contrast to the osteoblast-enriched ALCAM<sup>+</sup>Sca-1<sup>-</sup> population, the ALCAM<sup>-</sup>Sca-1<sup>+</sup> fraction contained cells capable of differentiating into both adipocytes and osteoblasts, suggesting either a mixed population of pre-adipocytes and pre-osteoblasts, or earlier mesenchymal bipotential progenitors. Consistent with this possibility, gene expression profiling indicated that this population most closely resembles PDGFR $\alpha$ <sup>+</sup>Sca-1<sup>+</sup> cells, recently identified by Morikawa *et al.* as containing mesenchymal stem cells (12). This population also expresses higher levels of cytokines and growth factors, including CXCL12. This finding is particularly interesting since Dr. Takashi Nagasawa's laboratory has proposed that a subset of stromal cells expressing CXCL12, or CXCL12<sup>hi</sup> reticular cells, found within the bone marrow and distinct from endosteal osteoblasts, provides crucial support for preproB cells (13;14). Whether these CXCL12<sup>hi</sup> reticular cells might share some overlap with ALCAM<sup>-</sup>Sca-1<sup>+</sup> or PDGFR $\alpha$ <sup>+</sup>Sca-1<sup>+</sup> cells, and represent mesenchymal stem or progenitor cells, awaits further clarification.

In summary, it has long been known that stromal cells provide support to hematopoietic development. Stromal cells are a mixed population, and recent studies have established that myriad components – osteoblasts, adipocytes, perivascular cells, and osteoclasts – can influence the HSC niche (4;6;15-17). Now the challenge facing this emerging field is to elucidate the relevant signals provided by each participant in this complex microenvironment. Historically, the field of skeletal biology has largely identified its cellular populations using anatomic techniques, while advances in hematopoiesis have been critically dependent on flow cytometry and use of cell-surface markers to identify subpopulations. In order to better understand the interactions of the skeletal and hematopoietic systems, and to take full advantage of pre-existing knowledge in both fields, the localization, immunophenotype, and functional properties of various cellular components within the bone marrow microenvironment must be reconciled. To that end, the current paper represents an important advance by defining mesenchymal cell populations that have distinct properties in supporting hematopoiesis, and offers new approaches to unanswered questions. Can ALCAM<sup>+</sup>Sca-1<sup>-</sup> cells support HSCs *in vivo*? Of note, single-cell Q-PCR analysis suggests that this is still a heterogeneous population, with a subpopulation that does not express osteoblast markers. Within the ALCAM<sup>+</sup>Sca-1<sup>-</sup> population, whether the osteoblasts or other cells are most critical remains unknown. Lineage tracing studies may address whether Sca-1<sup>+</sup> populations lose Sca-1 expression and then acquire ALCAM expression in a linear progression during osteogenic differentiation. Alternatively, do these subsets of stromal cells ultimately have unique fates within the bone marrow microenvironment? Given the limited number of HSC niches in the bone marrow, do ALCAM<sup>+</sup> cells give rise to all bone-forming osteoblasts, or do only a subset of endosteal “osteoblasts” provide HSC support? Tantalizing avenues for future investigation lie ahead.

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## References

1. 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 May 14. [Epub ahead of print]
2. Schofield R. The relationship between the spleen colony-forming cell and the haemopoietic stem cell. *Blood Cells*. 1978;4(1-2):7-25.
3. Taichman RS, Emerson SG. Human osteoblasts support hematopoiesis through the production of granulocyte colony-stimulating factor. *J Exp Med*. 1994 May 1;179(5):1677-82.
4. Calvi LM, Adams GB, Weibrecht KW, Weber JM, Olson DP, Knight MC, Martin RP, Schipani E, Divieti P, Bringham FR, Milner LA, Kronenberg HM, Scadden DT. Osteoblastic cells regulate the haematopoietic stem cell niche. *Nature*. 2003 Oct 23;425(6960):841-6.
5. Nilsson SK, Johnston HM, Coverdale JA. Spatial localization of transplanted hemopoietic stem cells: inferences for the localization of stem cell niches. *Blood*. 2001 Apr 15;97(8):2293-9.
6. Zhang J, Niu C, Ye L, Huang H, He X, Tong WG, Ross J, Haug J, Johnson T, Feng JQ, Harris S, Wiedemann LM, Mishina Y, Li L. Identification of the haematopoietic stem cell niche and control of the niche size. *Nature*. 2003 Oct 23;425(6960):836-41.
7. Visnjic D, Kalajzic Z, Rowe DW, Katavic V, Lorenzo J, Aguila HL. Hematopoiesis is severely altered in mice with an induced osteoblast deficiency. *Blood*. 2004 May 1;103(9):3258-64.
8. Wu JY, Purton LE, Rodda SJ, Chen M, Weinstein LS, McMahon AP, Scadden DT, Kronenberg HM. Osteoblastic regulation of B lymphopoiesis is mediated by Gsalpha-dependent signaling pathways. *Proc Natl Acad Sci U S A*. 2008 Nov 4;105(44):16976-81.
9. Zhu J, Garrett R, Jung Y, Zhang Y, Kim N, Wang J, Joe GJ, Hexner E, Choi Y, Taichman RS, Emerson SG. Osteoblasts support B-lymphocyte commitment and differentiation from hematopoietic stem cells. *Blood*. 2007 May 1;109(9):3706-12.
10. Chan CK, Chen CC, Luppen CA, Kim JB, DeBoer AT, Wei K, Helms JA, Kuo CJ, Kraft DL, Weissman IL. Endochondral ossification is required for haematopoietic stem-cell niche formation. *Nature*. 2009 Jan 22;457(7228):490-4.
11. Mayack SR, Wagers AJ. Osteolineage niche cells initiate hematopoietic stem cell mobilization. *Blood*. 2008 Aug 1;112(3):519-31.
12. 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 Oct 26;206(11):2483-96.
13. Nagasawa T. Microenvironmental niches in the bone marrow required for B-cell development. *Nat Rev Immunol*. 2006 Feb;6(2):107-16.

14. Tokoyoda K, Egawa T, Sugiyama T, Choi BI, Nagasawa T. Cellular niches controlling B lymphocyte behavior within bone marrow during development. *Immunity*. 2004 Jun;20(6):707-18.
15. Kiel MJ, Yilmaz OH, Iwashita T, Yilmaz OH, Terhorst C, Morrison SJ. SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. *Cell*. 2005 Jul 1;121(7):1109-21.
16. Kollet O, Dar A, Shivtiel S, Kalinkovich A, Lapid K, Sztainberg Y, Tesio M, Samstein RM, Goichberg P, Spiegel A, Elson A, Lapidot T. Osteoclasts degrade endosteal components and promote mobilization of hematopoietic progenitor cells. *Nat Med*. 2006 Jun;12(6):657-64.
17. Naveiras O, Nardi V, Wenzel PL, Hauschka PV, Fahey F, Daley GQ. Bone-marrow adipocytes as negative regulators of the haematopoietic microenvironment. *Nature*. 2009 Jul 9;460(7252):259-63.