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COMMENTARIES

Close Encounters of the Bone-Blood Kind

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Commentary on: 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 Oct 19;131(2):324-36.

A current "hot topic" in stem cell biology is identification of the presence and nature of the niche for stem cells in various tissues and organs, including the niche for hematopoietic stem cells (HSCs) (1). The first experimental support for bone marrow (BM) stroma serving as a specialized microenvironment or niche for long-term maintenance of hematopoiesis came almost 40 years ago from transplant experiments in which the hematopoietic microenvironment (HME) was transferred to an ectopic site upon *in vivo* transplantation of either BM fragments or BM stromal cells (BMSCs; (2-4)). Notably, the same transplant models provided support for the co-existence in BM of not only HSCs but also multipotent mesenchymal (bone, cartilage, fibrous tissue, adipocytes) progenitors, often designated the colony forming units-fibroblast (CFU-F), skeletal stem cells and/or mesenchymal stem cells (MSCs), amongst other monikers ((5), reviewed in (6)). Identifying these multipotent mesenchymal precursors has been difficult, however, for reasons spelled out below. Studies from Bianco, Robey and colleagues (7) now elegantly identify - anatomically, immunohistochemically and functionally - clonogenic skeletal progenitors with the capacity to self-renew and transfer the HME *in vivo*, while also linking their establishment and regeneration in BM to angiogenesis.

Although the presence of HSCs close to endosteal bone surfaces and a role for osteoblasts in supporting hematopoietic progenitors *in vitro* are well-accepted (reviewed in (8)), several issues contributed to uncertainty about the relationship between and exact nature of both the HSC niche in bone and the clonogenic, self-renewing BM stromal progenitors. These include the anatomic heterogeneity of BM stroma, which comprises several nonhematopoietic cell types (osteoblasts, endothelial cells, fibroblasts, and reticular cells), the documented functional heterogeneity of CFU-Fs from at least some species vis-à-vis support of specific hematopoietic lineages *in vitro* and osteogenic potential *in vitro* and *in vivo* (reviewed in (6)), and the lack of markers for unambiguous identification of specific stromal cell subtypes, including osteoblastic cells earlier in the lineage than mature osteoblasts and endothelial cells. The field took one leap forward several years ago with experimental evidence for a hematopoietic niche comprising osteoblasts on bone surfaces, *i.e.*, the so-called bone niche. Osteoblasts in the bone niche, however, have been variously characterized as parathyroid hormone (PTH)-activatable Jag1+ (Notch ligand jagged 1-positive) osteoblastic cells (9), a spindle-shaped N-cadherin+CD45- subset of osteoblastic (SNO) cells at the bone surface (10;11), and angiopoietin 1 (Ang1)+ osteoblasts regulating HSC number through the activation of the Tie-2/Ang1 signaling pathway (12). Over approximately the same period, a hematopoietic niche involving endothelial cells, the so-called vascular niche, was also described by use of SLAM family receptors that allowed anatomic localization of HSCs adjacent to sinusoids (specialized vessels that allow cells in venous circulation to extravasate into hematopoietic tissues) (13), raising some concerns or issues about how many and what kind of HSC niches might exist in BM. An important functional link between the vascular and endosteal/bone niches was uncovered about a year ago by Sugiyama *et al*. who demonstrated that the HSCs localized around sinusoids were usually in

contact with reticular cells that surrounded sinusoids and secreted high amounts of CXCL12 (also known as stromal-cell-derived factor (SDF)-1), a chemokine required for HSC maintenance (14). Interestingly, HSCs that localized to the endosteum were also usually adjacent to CXCL12-secreting reticular cells. However, how any of these populations or markers relates to multipotent stromal CFU-Fs remained unclear.

In a series of *in vitro* analyses and *in vivo* transplantation experiments, Sacchetti *et al*. demonstrate that a specific and regulatable phenotype defines the human BM stromal progenitors (BMSCs) that form all assayable CFU-Fs and their clonal progeny *in vitro*, regenerate bone and stroma and establish the HME *in vivo* (7). They first show that a subpopulation of CD45- non-hematopoietic BM cells highly express melanoma-associated cell adhesion molecule CD146 (also known as Mel-CAM or MCAM, P1H12, MUC18, A32 antigen, and S-Endo-1), a member of the immunoglobulin superfamily that functions as a Ca(2+)-independent cell adhesion molecule involved in heterophilic cell-cell interactions and is expressed in a restricted range of normal cells (15). In beautiful immunocytochemical and immunohistochemical analyses, the authors show that the $CD146^{high/bright}$ phenotype is shared by a subset of stromal cells *in vivo* – specifically subendothelial adventitial reticular cells of BM sinusoids – but not by endothelial cells, or osteoblastic cells that are capable of forming heterotopic bone but not an HME *in vivo*. Consistent with previous studies on the niche and their subendothelial nature, undifferentiated CD146+ BMSCs were found to express markers of early osteogenic progenitors but not of mature osteoblasts, and the HSC niche-related markers Jag1, N-cadherin, Ang1 and CXCL12 (9;10;12;14), and SCF (10). Very interestingly, by following the fate of transplanted CD146+ cells, including fates at the single clone level, during organogenesis of heterotopic BM, the authors document the capacity of at least some of the cells for stepwise regeneration into hematopoietic-supporting CD146+ subendothelial reticular cells in *de novo* BM, and conversely, for the transplants to regenerate CD146+ clonogenic CFU-Fs in culture. Again consistent with their origin from adventitial reticular cells, cultured CD146+ cells did not express endothelial markers or differentiate into endothelial cells under specific conditions. They did, however, express several markers of subendothelial cells (also called mural cells or pericytes, the latter a cell type with properties previously shown to be similar to MSCs/CFU-Fs), such as α-smooth muscle actin, NG2, calponin 1 and 3, and PDGFRβ. Overall, the data suggest self-renewal of CD146+ osteoprogenitors *in vivo* as an integral part of angiogenic events in which sinusoids are established prior to hematopoiesis and establishment of a hematopoietic niche.

As raised above, multiple different cell types in different anatomical locations in the BM and periosteum have been proposed previously as the *in vivo* counterpart of CFU-Fs/MSCs, with several putative markers (CD49a, CD63, CD90, CD105, CD140b, CD146, STRO-1, and alkaline phosphatase (ALP)). The new data provide a significant advance both in terms of confirming the utility of CD146 as a marker, at least for human CFU-Fs, with which to correlate observations *in vivo* and *ex vivo*, and in terms of characterizing the relationship of such cells to the HME. Establishing how well the CD45- $CD146^{high/bright}$ phenotype works as a marker for comparable populations in other species, including in mice where strategies not involving heterotopic HME-bone-BM formation may be used, will be interesting and important. The studies also advance the concept of and evidence for self-renewal of a mesenchymal stem or progenitor population, which previously was limited mainly to the capacity of stromal cell populations to undergo extensive passaging *in vitro*, with somewhat disparate data on retention of differentiation capacity with passaging, and no rigorous data on *in vivo* passaging of the sort available for, and part of the stem cell definition of, HSCs. Thus, the transplantation data that provide evidence for the ability of small numbers of CD146+ stromal cells, either as single CFU-Fs or pools of CFU-Fs, to function as self-renewing, clonogenic mulitpotent (giving rise to a minimum of osteoblasts and adventitial reticular cells, as shown in these studies) skeletal progenitors at least through secondary passage, are particularly notable. As the authors recognize, it will now be important to do additional studies to address issues such as serial passaging and the frequency within the CD146+ population of self-renewing clonogenic progenitors that are assayable *in vivo*, because estimates of

MSC and progenitor frequencies in BM have varied quite markedly in different studies, reflect largely analyses *in vitro* and have constituted a significant gap in rigorously defining the MSC phenotype. It is also worth considering whether and how the adherent CD146+ CFU-Fs relate ontologically to the non-adherent BM cell fraction described to have osteogenic (16) or both osteogenic and hematopoietic (17) potentiality and the circulating osteocalcin+ (or ALP+) osteoprogenitor cells identified in the peripheral blood (18). Also, although evidence for a mesenchymal cell lineage hierarchy has existed for many years (for review, see (6)), only a relatively few studies have queried bifurcation points for commitment and their regulation, for example, those for osteoblast-adipocyte precursors (reviewed in (19)) and for osteoblast-chondroblast precursors (20). Thus, regulation of fate choices that underlie restriction of multipotent CFU-Fs and other osteoprogenitor pools remains largely unexplored and hampered by the paucity of markers already noted (see, however, (21;22)). It is therefore also of interest that Sacchetti *et al*. provide evidence that $CD146^{low/dim}$ cells originating from other/non-BM anatomic compartments of bone (*i.e*., trabecular bone or periosteum) form differentiated osteoblasts and bone when transplanted *in vivo* but do not transfer the HME, while $CD146^{high/bright}$ clonogenic BM progenitors form bone and transfer the HME *in vivo*, but the two functions can be experimentally dissociated by FGF-2 treatment. It will, therefore, be of great interest to combine additional cell fractionation strategies, *e.g*., on the basis of CD146 expression and expression of other markers, with other kinds of manipulations, including the effects of hormones and growth factors on developmental and functional endpoints.

The data of Sacchetti and colleagues (7) support the view that less mature cells, *i.e*., osteoprogenitors, rather than mature osteoblasts, comprise the endosteal niche. This is consistent with the previous characterization of osteoblastic cells in the bone niche as "early" rather than "late" osteoblasts (10) and with the possibility that PTH expansion of the bone niche (9;23) may reflect, at least in part, the ability of PTH to increase the pool of differentiating osteoprogenitors (reviewed in (24)). They also extend the functional link proposed by Sugiyami *et al*. (14) and suggest that the bone/endosteal and vascular niches may not be so different, and indeed may be one and the same, or at least derive from the same progenitors. On the other hand, the dynamic stepwise regeneration of the HME, skeletal progenitors associated with BM sinusoids and angiogenesis as described in the transplants (7), together with other recent data on the establishment and turnover of HSCs and other niches, underscores the need for acceleration of studies to identify additional markers for $CD146^{high/bright}$ cells/MSCs/CFU-Fs, their progeny, and other stromal populations, as well as for analysis of the likely distinct nature of niches for quiescent versus cycling and differentiating HSCs. They also indicate that simultaneous analysis of the frequencies of HSCs and MSC/CFU-Fs and their progeny in more mouse models with genetic modifications affecting osteoblasts/bone and samples from humans with mutations affecting osteoblasts/bone and other stromal populations (reviewed in (1;25)), as well as analysis of mice and humans treated with agents down- or up-regulating osteoblast lineage cells, including, *e.g*., PTH (23), strontium (11) and others, will not only increase understanding of basic developmental paradigms governing the bone and blood lineages, but also help to advance therapeutic options for a variety of disease states including hematopoietic disorders and cancer.

Conflict of Interest: None reported.

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