Mesenchymal Stem Cell: Keystone of the Hematopoietic Stem Cell Niche and a Stepping-Stone for Regenerative Medicine

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Abstract

Mesenchymal stem cells (MSCs) are self-renewing precursor cells that can differentiate into bone, fat, cartilage, and stromal cells of the bone marrow. Recent studies suggest that MSCs themselves are critical for forming a niche that maintains hematopoietic stem cells (HSCs). The ease by which human MSC-like and stromal progenitor cells can be isolated from the bone marrow and other tissues has led to the rapid development of clinical investigations exploring their anti-inflammatory properties, tissue preservation capabilities, and regenerative potential. However, the identity of genuine MSCs and their specific contributions to these various beneficial effects have remained enigmatic. In this article, we examine the definition of MSCs and discuss the importance of rigorously characterizing their stem cell activity. We review their role and that of other putative niche constituents in the regulation of bone marrow HSCs. Additionally, how MSCs and their stromal progeny alter immune function is discussed, as well as potential therapeutic implications.

INTRODUCTION AND A NOTE ABOUT NOMENCLATURE

Mesenchymal stem and progenitor cell (MSPC) therapy has great therapeutic potential but remains largely uncharacterized. The idea that precursor cells—capable of forming bone—exist in bone marrow (BM) arose from seminal studies by Friedenstein and colleagues (1), who rigorously established that suspensions of dispersed BM cells could form fibroblastic colonies (colony-forming unit fibroblasts, CFU-F) that were derived from single cells. These investigators felt that CFU-F represented the alter ego of colonies that form in the spleen (CFU-S)—the hematopoietic progenitors identified by Till & McCulloch (2)—which exhibited striking similarities to CFU-F in their recovery from whole-body irradiation (3). Most importantly, self-renewal and differentiation potential were demonstrated by in vivo transplantation experiments (1, 4). These classic papers formed the basis for the suggestion that genuine stromal stem cells exist in the marrow.

By extension from embryonic mesenchymal cells, Caplan (5) coined the term "mesenchymal stem cells" in 1991 to refer to adult BM precursors of bone, cartilage, and other mesodermal tissues and predicted that these so-called MSCs would represent a major arsenal in self-cell therapy for regenerative purposes. This prediction was prescient, given the ensuing explosion of publications regarding the use of cultured MSCs, which led to 220 clinical trials worldwide that are currently testing the various clinical virtues of MSCs (**Table 1**).

The ease by which human BM-derived MSPCs could be culture-expanded and then differentiated into multiple lineages led to questions about the homogeneity of cultured cells and concerns about how many of these cultured stromal cells could in fact be stem cells. The International Society for Cellular Therapy (ISCT) encouraged the scientific community to use the term "multipotent mesenchymal stromal cells" when stem cell activity was not clearly demonstrated (6). The group felt it was important to keep the acronym "MSC" because it had

Table 1 Clinical trials (registered as of June 2, 2012) using mesenchymal stem cells

Indication	Number of studies
Immunomodulation	**48**
Multiple sclerosis/atherosclerosis	12
Type 1 diabetes	12
Crohn's disease	10
Systemic lupus erythematosus/colitis	4
Rheumatoid arthritis/Sjögren's syndrome	3
Buerger's disease/sickle cell disease	2
HIV	1
Limbus corneae insufficiency syndrome	1
Periodontitis	1
Progressive hemifacial atrophy	1
Retinitis pigmentosa	1
Tissue protection	**76**
Myocardial infarction/stroke/ischemia	34
Liver cirrhosis	20
Alzheimer's/Parkinson's disease	4
Amyotrophic lateral sclerosis	4
Fibrosis/emphysema	4
Necrosis	4
Acute kidney injury	2
Bronchopulmonary dysplasia	2
Multiple system atrophy/multiple trauma	2
Regenerative medicine	**69**
Osteoarthritis/osteogenesis imperfecta	22
Bone/cartilage repair	18
Spinal cord injury/neuroblastoma	8
Anemia	4
Type 2 diabetes	4
Dilated cardiomyopathy	4
Wound healing/umbilical cord varices	3
Ataxia	2
Autism	1
Epidermolysis bullosa	1
Erectile dysfunction	1
Wilson's disease	1
Graft enhancement	**27**
GvHD	23
Hematopoietic malignancies	4

been used in the literature for decades. However, the use of the same acronym to designate two distinct entities is bound to engender confusion and likely to limit our ability to define bona fide stem cells. In this article, we use the acronym MSPC, by analogy to the hematopoietic system, when the population is undefined but expected to contain mesenchymal stem and progenitor cells, and we restrict the use of MSC, as mesenchymal stem cells, for populations that have been demonstrated to enrich for stem cell activity. This article reviews recent advances in the characterization of MSCs, their properties for forming hematopoietic niches, and their abilities to regulate the immune response and coordinate tissue regeneration. Several excellent reviews have recently been published on these topics (7–18). The reader is invited to consult them for additional information as they are referred to more specifically upon discussion of these subjects.

IDENTIFICATION AND CHARACTERIZATION OF MSCs

Surface Markers

With the emergence of surface antigens marking culture-expanded MSPCs (19), the ISCT proposed minimal criteria to define human MSCs (as multipotent mesenchymal stromal cells) in a second position statement (20). By these criteria, multipotent mesenchymal stromal cells were defined as plastic adherent cells that must express CD105, CD90, and CD73 but that lack the expression of pan-leukocyte (CD45), endothelial, or primitive hematopoietic (CD34), monocytic (CD14 or CD11b), or B cell (CD79a or CD19) markers, as well as lack the expression of HLA class II (HLA-DR) surface antigen, which is not found on steady-state MSPCs. Additionally, bulk cell populations must show tri-lineage differentiation into osteoblasts, adipocytes, and chondroblasts. Although these criteria represented a helpful step to standardize cell preparations for applied research, it did little to help dissect the nature of these cells or to resolve the confusion in the literature. If one enters "mesenchymal stem cells" in a PubMed search, >18,000 articles are retrieved, of which only a handful have rigorously tested "stemness" of the cell preparation (**Table 2**). Defining stem cells and their progeny will be essential to improving on existing cell therapy. Significant biological effects have been documented after injection of multipotent mesenchymal stromal cells, but it remains unknown whether a stem cell, a committed progenitor, or another stromal cell can mediate these effects.

Of the three positive surface antigens suggested by the ISCT, only CD105 was shown to be expressed on fresh (uncultured) human MSPCs, which as a bulk population could give rise to CFU-F (21). However, we know of no published evidence that CD105 is expressed on MSCs that are clonally multipotent and capable of self-renewing in vivo. Nor has CD73 and CD90 expression been prospectively evaluated on native MSCs. The importance of prospective isolation was highlighted by the reported changes in the expression of the adhesion protein CD44, a marker highly expressed on in vitro–expanded MSPCs (19, 22, 23) but recently shown to be acquired in culture. Freshly sorted mouse and human BM stromal $CD44^+$ cells indeed exhibited little or no CFU-F activity, whereas the $CD44^-$ fraction contained almost all the clonogenic cells with multilineage differentiation potential (24). Likewise, CD146 (melanoma-associated cell adhesion molecule) was shown to be upregulated in culture and downregulated when cells were cultured under hypoxic conditions (25). CD105 expression was also enhanced on adipose-derived adherent stromal cells during cultivation (26). Changes in the expression of some surface markers during cell culture may coincide with in vitro differentiation. This appears to be the case, for example, for STRO-1 or CD271, markers shown to enrich for human CFU-F activity (27–30) or Nestin expression in mouse MSCs (31), which are downregulated after cell cultivation.

Only a few surface markers have thus far been shown to define MSCs (**Table 2**). In a set of elegant studies, Bianco and colleagues

Table 2 Human and mouse bone marrow MSC markers tested in vivo

Cell phenotype (Reference)	Clonogenicity (CFU-F/ spheres)	In situ detection	In vivo self-renewal (≥2 trans–plantations)	In vivo contribution to bone marrow microenvironment
Mouse				
Nestin^{+}CD45^{-}CD31^{-} (31)	+	+	+	+ (clonally expanded cells)
CD51^{+}CD105^{+}CD90^{-}CD45^{-}Tie2^{-} (33)	nd	nd	nd	+ (multiclonal freshly isolated cells)
PDGFRα^{+}Sca1^{+}CD45^{-}Ter119^{-} (34)	+	+	−	+ (multiclonal freshly isolated cells)
Human				
CD146^{+}CD45^{-} (32)	+	+	+	+ (clonally expanded cells)
STRO-1^{Bright}CD146^{+}(70)	+	+	nd	+ (multiclonal, ex vivo expanded cells)
STRO-1^{Bright}CD106^{+} (29)	+	nd	nd	+ (clonally expanded cells)
SSEA4^{+} (44)	+	nd	nd	+ (multiclonal, ex vivo expanded cells)
CD146$^{+/-}$CD271^{+}Lin^{-}CD45^{-} (25)	+	+	nd	+ (multiclonal, ex vivo expanded cells)

nd, not determined.

(32) showed that human CD146^{+}CD45^{-} expression marked self-renewing osteoprogenitor cells containing all the BM CFU-F activity and capable of generating a heterotopic BM niche in a subcutaneous transplantation model. More recent studies have suggested that a similar frequency of CFU-F could be recovered from CD271^{+}CD146$^{-/low}$CD45^{-} human BM cells (25). In the mouse, the CD51^{+}CD105^{+}CD90^{-}CD45^{-}Tie2^{-} subset of the fetal bone was capable of endochondral ossification and reconstitution of hematopoietic activity upon injection under the kidney capsule (33). In the adult mouse BM, PDGFRα^{+}Sca1^{+}CD45^{-}Ter119^{-} perivascular cells could give rise to osteoblasts, reticular cells, and adipocytes in vivo upon transplantation into irradiated mice (34). Other independent studies have revealed that the intermediate filament protein Nestin marked perivascular stromal cells (Nestin^{+}CD31^{-}CD45^{-}) that contain all the CFU-F activity within the BM and the exclusive capacity to form clonal spheres (termed mesenspheres) when cultured in nonadherent conditions (31). Serial transplantation analyses revealed that Nestin^{+} clonal mesenspheres can self-renew and generate hematopoietic activity in heterotopic bone ossicle assays (31). Because only a fraction of CFU-F represents genuine MSCs, much work remains to be done to define MSCs and distinguish them from differentiated progeny.

Multipotency

A major defining characteristic of MSPCs is their ability to differentiate into the three major mesenchymal lineages—bone, cartilage, and adipose tissues (**Figure 1**)—upon culture under specific in vitro conditions (19). MSPCs also give rise to BM stromal cells that promote hematopoiesis (35), although the nature of these stromal cells remains obscure. Multipotency must be evaluated using clonal assays rather than bulk populations. Nonclonal assays are not interpretable because they cannot prove that the different lineages arose from the same progenitor. Regrettably, most publications on MSPCs have thus far reported results on tri-lineage differentiation potential using polyclonal populations.

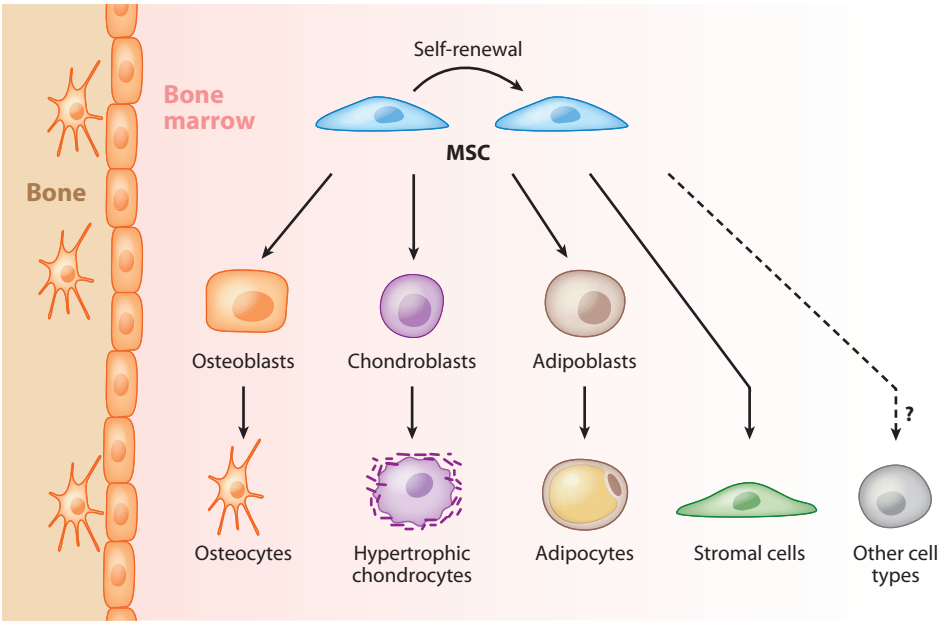

Figure 1

Bone marrow (BM) mesenchymal stem cells (MSCs) give rise to cells forming the skeleton. BM-resident MSCs self-renew, giving rise to identical cells that may differentiate toward the lineages that form the skeleton and BM stroma. MSC-derived mineralizing osteoblasts are embedded in the bone matrix to become osteocytes, although chondroblast and adipoblast precursors can give rise to cartilage-forming hypertrophic chondrocytes and fat-storing adipocytes, respectively. Differentiation into other mesodermal and nonmesodermal cell types has been reported but remains controversial.

Importantly, the culture of mouse MSCs is much more challenging than that of human MSPCs. Isolation of mouse MSCs by adherence to plastic is fraught with difficulties given their low frequency and contamination with hematopoietic cells (36). To overcome these hurdles, new techniques have been developed using retroviral selection (37), culture in nonadherent conditions (31), isolation of BM "plugs" (38), and use of compact bone as an enriched source of MSPC activity (39).

In addition to the classical tri-lineage potential, MSPCs may also differentiate into other mesodermal or even nonmesodermal cell types, such as myoblasts, hepatocytes, and neural cells (23, 40–43). Interestingly, adult BM MSPCs express the SSEA4 marker, a glycolipid antigen commonly used to identify undifferentiated human pluripotent stem cells that can differentiate toward all tissues. However, SSEA4$^+$ MSPCs were clonally tested only for bone, cartilage, and fat differentiation, and thus far there is no evidence of pluripotency for these cells (44). The capacity of MSPCs to differentiate into other lineages remains controversial, however, and may, at least in some cases, arise from spontaneous cell fusion events of donor transplanted MSPCs with recipient cells from other tissue origins (45). Although adult MSCs are generally considered mesodermal in origin (46), lineage tracing studies have revealed that endochondral bones of the head and shoulder have a neural crest origin (47, 48). BM-derived MSPCs express neuroectoderm lineage markers such as CD271 (nerve growth factor receptor) and Nestin, and they can form spheres that resemble neurospheres. However, the transcriptional program of adult MSCs appears to be distinct from that of adult neural stem cells and of many other stem cell types

(31). Thus, even though MSPCs can be forced to adopt neural features in specific culture conditions (49), there is little evidence of a physiological relevance.

MSPC activity can be recovered from many organs during fetal development (50, 51) and also in adults (43, 52–55). Adipose tissue–derived multipotent stromal cells have gained popularity because they are relatively easy to access and because of the expanding use of cosmetic liposuction procedures. However, it is becoming clear that marrow and extramedullary stromal cells are not interchangeable. For example, both adipose-derived and dermis-derived stromal progenitors show tri-lineage differentiation in vitro but also show significant differences in their transcriptome profiles (56). Whereas adipose-derived stromal cells can be induced to differentiate into mineralizing osteoblasts in vitro as efficiently as BM-derived MSPCs (57), they cannot robustly form bone in vivo by heterotopic transplantations unless preconditioned by cytokines or specific media (58–60). Perivascular PDGFRβ$^+$ (platelet-derived growth factor receptor beta) mural cells in the adipose tissue do contain progenitors for adipocytes, however (61). Likewise, CD146$^+$ human skeletal muscle pericytes exhibit myogenic progenitor activity but cannot form bone in vivo unless stimulated by bone morphogenetic protein (BMP)-2 (62, 63). Thus, several adult stem cell types localize in vascular niches of multiple tissues, including BM (MSCs and HSCs) (31), fat (61), brain (64), and skeletal muscle (62), but there is no firm evidence that the perivascular progenitors can form organs other than their own tissue of origin.

That perivascular cells contain "undifferentiated mesenchymal cells" has long been suspected (65, p. 50). Pericytes, also referred to as mural cells, lie on the abluminal side of blood vessels, immediately apposed to endothelial cells, and exhibit MSPC characteristics in vitro (66). Cultured pericytes can differentiate into osteoblasts, adipocytes, and chondroblasts, as well as into smooth muscle and skeletal muscle cells (62, 67–69). In addition, pericytes isolated from various tissues and human BM MSPCs share a similar expression profile of cell surface antigens such as CD146, NG2 (chondroitin sulfate proteoglycan 4), PDGFRβ, and cytoplasmic α-smooth muscle actin (29, 32, 43, 70, 71). They also display similar morphological characteristics in culture (32, 43). However, no marker has thus far been shown to be specific for pericytes or MSPCs, and all markers currently used appear dynamic in their expression profiles according to the developmental stage, in vitro culturing, ongoing pathological situation, or species of origin (reviewed in 7, 8). To what extent MSCs and pericytes overlap biologically and functionally remains unclear at present. Although a classical defining criterion for pericytes is their association with the microvasculature (capillaries, postcapillary venules, and terminal arterioles) (8), MSPC-like activity has been found in the walls of larger vessels such as arteries and veins (72, 73). In addition, MSPC activity has been isolated from cartilage tissues lacking vascularization (74). Thus, not all pericytes appear to be MSCs, nor are all MSCs likely to be pericytes.

Self-Renewal

As is the case for any stem cell, self-renewal is a major criterion required for MSC identification. Thus far, the CFU-F assay remains a major tool used to determine clonogenicity of progenitors and stem cell activity. Clonal CFU-F have been expanded in culture and then serially transplanted into immunodeficient recipients to demonstrate self-renewal (32). Alternatively, another assay was developed in which stromal cells (CD45$^-$Ter119$^-$CD31$^-$) or FACS-purified subsets were shown to form mesenspheres when plated in nonadherent culture conditions adapted from neural crest (75), pericyte (76), and MSC (31) culture (**Figure 2**). Single mesenspheres can self-renew in vitro and robustly in vivo after serial transplantation (31). Importantly, recent analyses suggest that mesenspheres can also be recovered from the stromal fraction of human BM (S. Pinho and P.S. Frenette, unpublished data). However, it is currently not clear how CFU-F relates to

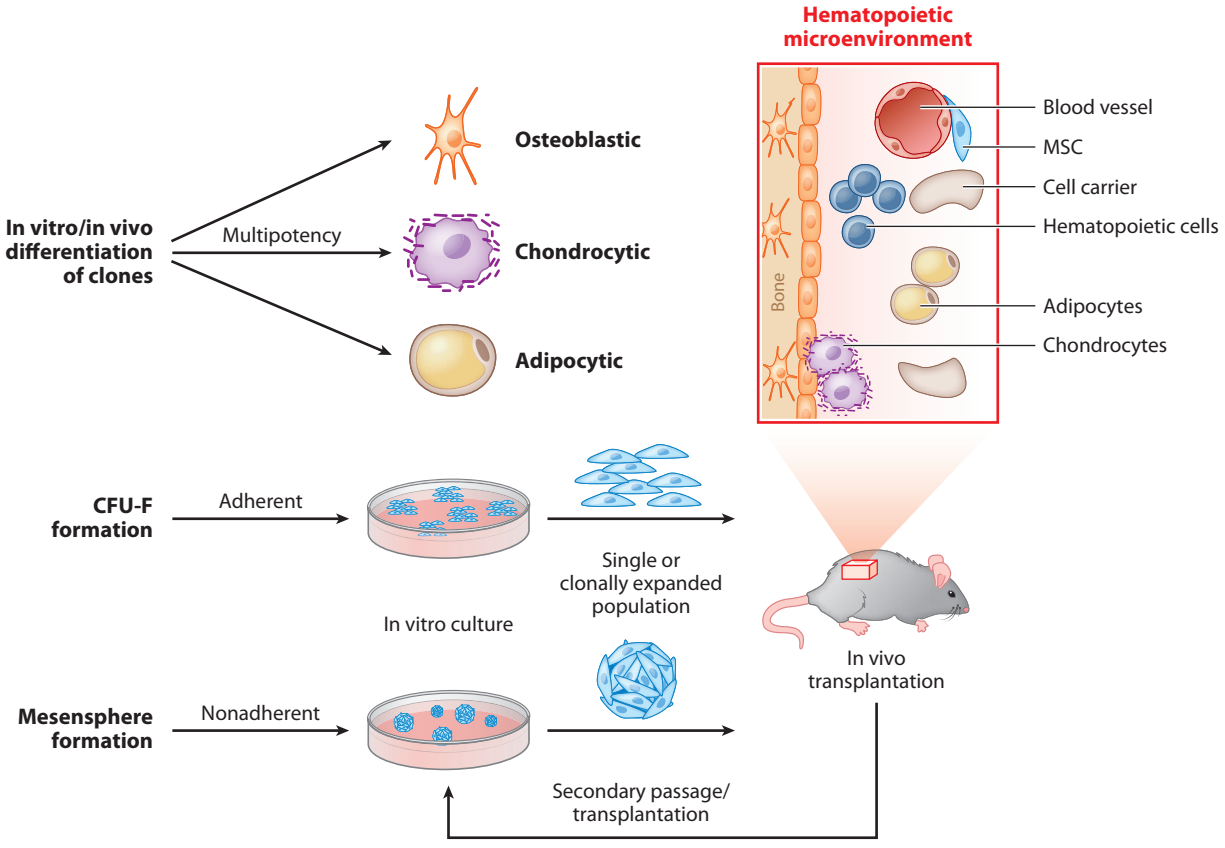

Figure 2

Functional assays to characterize MSCs. To characterize genuine MSCs, stringent clonal assays demonstrating multilineage differentiation and self-renewal in vitro and, most importantly, in vivo should be employed. Standard protocols exist to induce MSPC differentiation toward the osteoblastic, chondrocytic, and adipocytic lineages. In vivo differentiation assays can be performed by transplantation of bone marrow (BM) MSPCs under the renal capsule or in subcutaneous pockets. Cells can be adsorbed using different scaffolds, such as hydroxyapatite/tricalcium phosphate particles, or using denatured collagen sponges. Upon in vivo transplantation, bona fide MSCs contribute to ectopic formation of an organized hematopoietic microenvironment where BM stromal cells and active hematopoiesis can be detected. In culture, MSPC clonogenicity can be determined by adherent colony-forming unit fibroblast (CFU-F) and nonadherent mesensphere assays. Clonally expanded CFU-F and/or single mespheres (or clonally expanded mespheres) can be serially transplanted in vivo, demonstrating their self-renewal capacity without losing their multipotent mesenchymal potential.

mespheres. The fact that many more cells were required to observe self-renewal from expanded CFU-F ($\sim 10^6$ cells) compared with a single mesensphere ($\sim 10^3$ cells) (31, 32) suggests that more primitive MSCs are preserved in the spheres than in CFU-F. This possibility needs to be evaluated experimentally.

Although it is clear that in vivo transplantation of clonal populations should define authentic MSCs, the duration of reconstitution required to conclude that stem cell activity is present in the tested cell preparation remains undefined. In the hematopoietic system, it is generally accepted that long-term repopulating HSCs should be measured at least 16 weeks after transplantation. This has been based on the notion that short-term progenitors disappear 3–4 months following transplantation (77–79); hence, the detection of donor progeny at 16 weeks must mean that they have originated from transplanted HSCs. Although the skeleton is generally thought to turn over

much more slowly than the BM, recent elegant genetic studies using transgenic mice to label osteoblasts and osteoprogenitors (marked by osteocalcin and osterix promoters, respectively) have revealed, unexpectedly, that 90% of osteoblasts had turned over in 25 days and that cells labeled with the osterix promoter disappeared after 90 days (80). Whether half-life in homeostasis can be translated to assays using heterotopic ossicles remains to be evaluated. These studies, however, raise the possibility that the kinetics of hematopoietic and osteogenic progenitors may be similar in mice.

THE MSC AND ITS STROMAL DESCENDANTS IN THE HSC NICHE

Owen (65, 81) predicted in the mid-1980s that the stromal constituents in the BM were hierarchically organized just like the hematopoietic system. Although this idea remains very much alive over 25 years later, the road ahead is long, as little new knowledge has been gained in the interim. However, there is reason for optimism with the development of specific genetic models that will likely yield important new insights in the next few years. Much of the recent research has focused on the top of the hierarchical tree, with the identification and characterization of the HSC niche.

The Niche Concept

The concept of a niche was proposed by Schofield (82; reviewed in 17), referring to a regulatory unit that maintains and directs HSC self-renewal and differentiation. This idea was supported by experimental studies in model organisms such as *Caenorhabditis elegans* and *Drosophila*, where specific niche cells maintain germ-line stem cells. Whether a similar niche exists in the mammalian BM is under active study. If a stem cell niche exists, then several basic criteria would be expected from stromal cell niche candidates:

1. Rarity. HSCs are very rare cells in the BM, comprising 0.005–0.01% of BM nucleated cells. Because the HSC niche is highly regulated and saturable (83), genuine niche cells are expected to be extremely rare in the BM.
2. Physical proximity. The development of sophisticated imaging technologies and genetic markers have made possible the precise determination of anatomical relationships between cells or structures in the BM (31, 84–87). Using histological sections or intravital microscopy, distances between endogenous HSCs or exogenously purified and transplanted HSCs and stromal cells can readily be measured. Each method, however, has its own drawbacks: The use of histological sections provides only a two-dimensional view of the BM and limits the assessment of structures that are above or below the section plane, leading to possible overestimation of distances between HSCs and the nearest niche cell. Because there is so far no known fluorescent marker that can specifically track endogenous HSCs, intravital microscopy imaging has been done with FACS-purified HSCs that are injected in steady-state or irradiated recipients, a technique potentially prone to artifactual observations. These limitations highlight the need to label endogenous HSCs in their native tridimensional environment.
3. Synthesis of HSC maintenance genes. Several factors influence HSC function, such as the chemokine CXCL12, which regulates HSC migration to and out of the BM (88); stem cell factor (SCF, also called kit ligand), which controls HSC quiescence and adhesion (89); Angiopoietin-1, Osteopontin, and Thrombopoietin-1, which contribute to maintaining HSC quiescence (90–94); vascular cell adhesion molecule-1 (VCAM-1), which mediates HSC adhesion (95, 96); and Notch ligands, which contribute to HSC maintenance and self-renewal (97, 98).

4. Selective regulation of HSC function by the niche. The HSC niche must sense the need to induce HSC migration, division, or differentiation, and thus one would expect niche cells to be regulated differently from other BM stromal cells and to support other hematopoietic functions. One example is the long-range adrenergic signals from the sympathetic nervous system (SNS) delivered to the HSC niche, signals that regulate circadian HSC egress from BM (99).

Identification of Candidate Niche Cells

Several assays have been used to identify and characterize candidate niche cells. Imaging techniques can reliably identify HSCs in their native environment, and thus the relationship with various stromal structures can be assessed, keeping in mind the aforementioned limitations. The time-tested heterotopic transplantation assay represents a gold-standard functional test in which FACS-purified candidate stromal cells are embedded in a matrix that prevents their diffusion and are then transplanted into recipient mice (usually subcutaneously or under the kidney capsule). Several weeks later, the transplanted area can be examined for the presence of heterotopic bone that is assayed through immunohistochemistry/FACS for the presence of hematopoiesis of host origin supported by a stroma of donor origin (**Figures 2** and **3*a***). This type of assay has been used to characterize human $CD45^{-}CD146^{+}$ cells (32), murine fetal $CD105^{+}CD51^{+}CD90^{-}CD45^{-}Tie2^{-}$ cells (33), and $Nestin^{+}$ cells (31) as putative niche cells. The main limitation of this assay is that it cannot determine whether the transplanted cells indeed are niche cells or instead are more immature progenitors that can differentiate into niche cells.

As is often the case in biology, the most precise and definitive methods are genetically based. Genetic approaches may limit the available models that allow specific genetic manipulation in the desired population. Three different types of tools have been employed (**Figure 3*b–d***), one of which is to genetically manipulate the cells to expand or activate the putative niche population in vivo and then to assess the impact on HSC numbers (**Figure 3*b***) (97, 100). In a second assay, specific expression of a suicide gene such as thymidine kinase or the diphtheria toxin receptor protein can be used to deplete the niche cell and evaluate the impact on HSCs and hematopoiesis (**Figure 3*c***) (31, 101, 102). The main limitation of these assays is that they do not account for the possibility that the targeted cells might not regulate HSCs directly. In addition, nonspecific bystander effects evoked by the loss of a population of stromal cells might theoretically obscure the experimental readout. The most specific and elegant genetic assay is to delete specific genes in a specific niche cell candidate to test function (**Figure 3*d***) (103). This approach's advantages are that (*a*) it does not affect other components of the hematopoietic niche and (*b*) it allows a much more detailed dissection of the contribution of the target population to total niche activity by looking at both the specific molecules and cell types involved. Its main disadvantages are that the readout will be as good (or as poor) as the reporter used for targeted deletion and that some actual niche populations might be overlooked owing to compensatory effects by other cells.

DISSECTING THE MESENCHYMAL CONSTITUENTS OF THE HSC NICHE

Several cell types may contribute to the HSC niche, incrementally raising its apparent complexity (**Figure 4**). Early BM fractionation studies suggested that HSCs and immature progenitors were enriched in the marrow closest to the bone (endosteal region) (104). In addition, studies in which HSC-enriched populations were transplanted in nonmyeloablated recipients suggested that hematopoietic progenitors were preferentially located near the bone surface (84). These studies thus

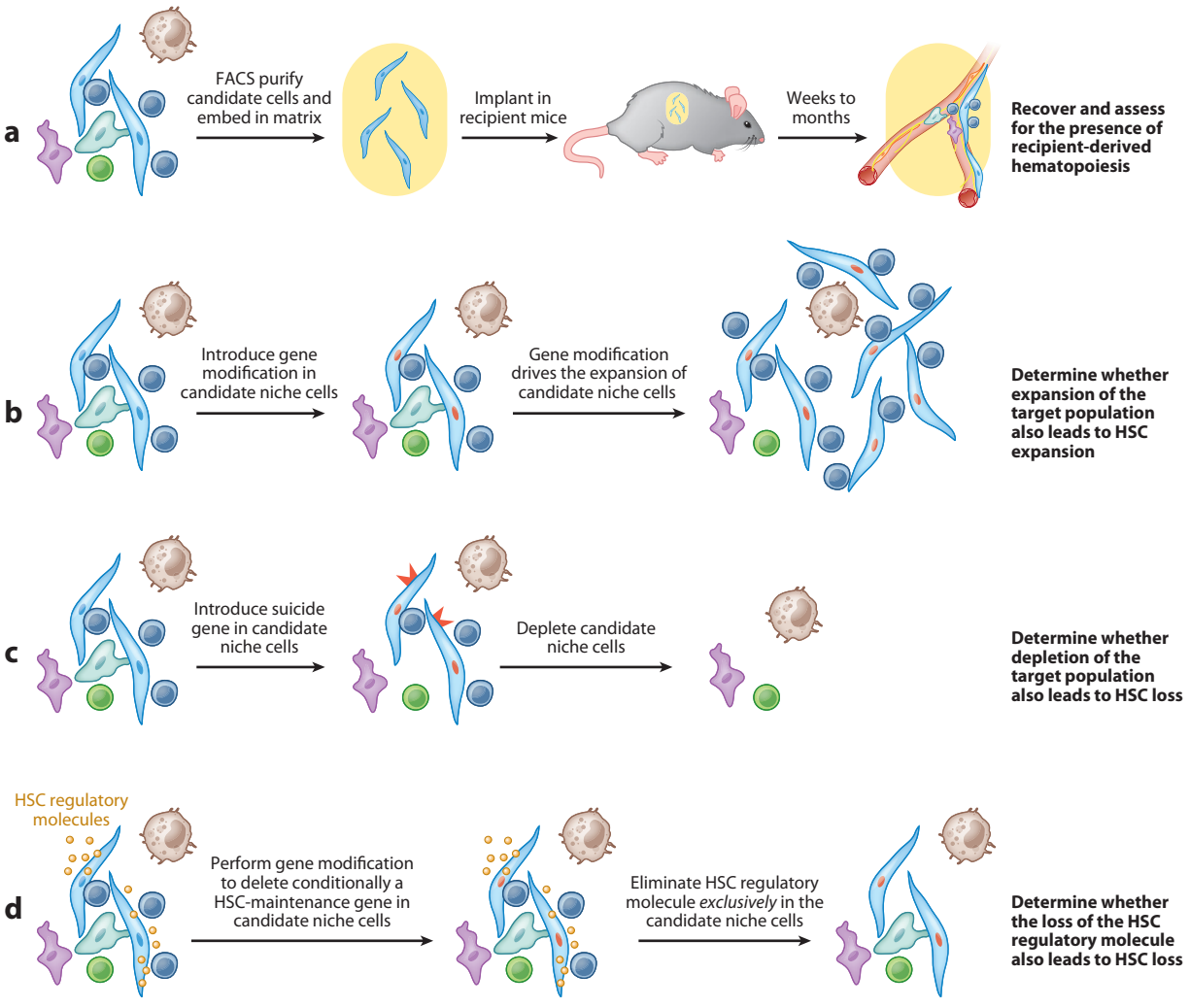

Figure 3

In vivo assays to determine niche function. (*a*) Heterotopic transplantation assay. In this assay the candidate cells are transplanted into recipient mice to test their ability to generate hematopoietic niches. (*b*) Expansion of niche cells. Specific gene manipulation leads to an increase in the number of niche cells, which in turn can result in HSC expansion. (*c*) Deletion of putative niche cells. Specific gene manipulation can be engineered for the conditional depletion of the desired niche population, which would be expected to result in HSC reduction. (*d*) Conditional deletion of HSC maintenance genes. Genetic deletion of specific candidate HSC regulatory molecules in the desired niche population may lead to HSC loss from the BM. (Abbreviations: BM, bone marrow; FACS, fluorescence-activated cell sorting; HSC, hematopoietic stem cell.)

indicated the existence of an endosteal niche located close to the bone. Osteoblasts were an obvious candidate niche cell because they were reported to support short-term HSC expansion in vitro and to secrete multiple factors that can regulate HSC activity (105, 106). Further studies using improved genetic and imaging techniques further strengthened the notion that osteoblast lineage cells were playing a role. In one study by the Scadden lab (97), the authors generated mice in which the Collagen α1 promoter directed the expression of a constitutively activated parathyroid hormone receptor specifically in osteoblastic lineage

cells. In these mice, an increase in the number of osteoblasts correlated with increased numbers of HSCs (97). A similar phenotype was detected when wild-type mice were injected with recombinant parathyroid hormone (97). In an accompanying study by the Li laboratory (100), the authors generated *Mx1–Cre; Bmpr1a*$^{\Delta/\Delta}$ mice in which PolyI:C injection led to the deletion of the BMP receptor 1a (*Bmpr1a*) gene in both hematopoietic and stromal cells (100). The authors found that this genetic deletion rapidly increased osteoblast and HSC numbers in the BM. The HSC expansion was not due to a cell-autonomous effect of *Bmpr1a* deletion, given that transplantation of wild-type cells into *Bmpr1a*$^{-/-}$ mice also led to expansion of the donor HSCs (100). Loss-of-function studies using depletion of osteoblast-lineage cells in transgenic mice expressing herpes virus thymidine kinase under the Collagen α1 promoter revealed a major alteration in hematopoiesis but only a mild effect on the HSC-enriched fraction (Lin$^-$Sca-1$^+$c-kit$^+$ cells) (102).

Other studies had found that during HSC mobilization induced by granulocyte colony-stimulating factor (G-CSF), osteoblastic function was dramatically suppressed (107, 108), further implicating osteoblastic lineage cells in the regulation of the HSC niche, although the data were correlative. However, additional reports showed that changes in osteoblast numbers do not necessarily cause changes in HSC numbers. Mice lacking biglycan had reduced numbers of bone-lining osteoblasts (109), and mice treated with strontium had higher numbers of osteoblasts, but in both cases HSC numbers in the BM

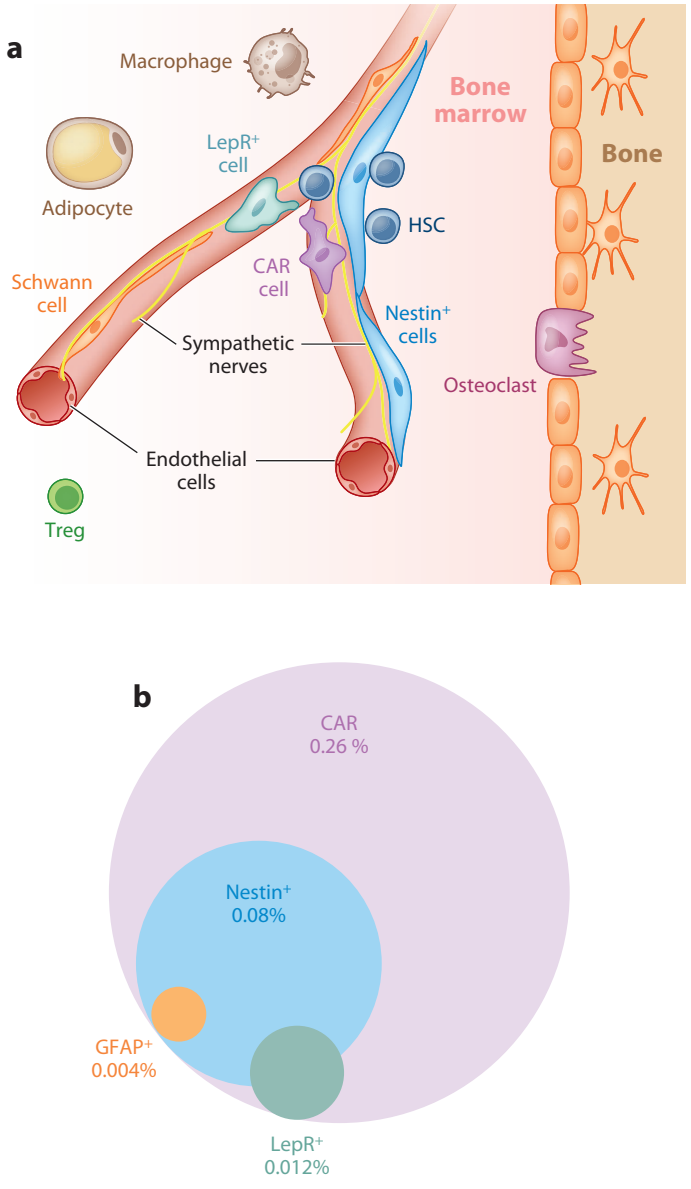

Figure 4

The evolving hematopoietic stem cell niche. (*a*) Schematic representation of the HSC niche in the bone marrow as well as niche accessory cells. The niche benefits from a close interrelationship between endothelial cells and perivascular cells; both cell types synthesize soluble and cell-contact factors that promote stem cell maintenance. Accessory cells, such as CD169$^+$ macrophages, adipocytes, osteoclasts, and regulatory T cells (Tregs), regulate niche cells, thus indirectly contributing to stem cell maintenance. (*b*) Perivascular cells comprise, thus far, CXCL12-abundant reticular (CAR) cells, Nestin$^+$ MSCs, nonmyelinating Schwann cells, and LepR$^+$ cells that are found at different frequencies and display some overlap. Note that the frequency of CAR, Nestin$^+$, and LepR$^+$ cells was quantified by flow cytometry, whereas the frequency of glial fibrillary acidic protein (GFAP$^+$) cells was determined by immunofluorescence and thus may have been underestimated.

were unaffected (109, 110). Thus, these studies suggested (*a*) the presence of HSC niches in the murine BM and (*b*) that osteoblast lineage cells were a component of this niche but may not be required for HSC maintenance in the BM.

Microarray analyses revealed differential expression of the SLAM markers (CD150 and CD48) on HSCs and committed progeny. $CD150^+CD48^-CD41^-$ expression was highly enriched for HSCs that were localized near sinusoidal endothelium in the BM and spleen, suggesting the existence of a perivascular niche (87). Mice in which the green fluorescent protein (GFP) was knocked in the *Cxcl12* locus identified a population of perivascular reticular cells (named CAR cells for CXCL12-abundant reticular cells), some of which were associated with $CD150^+CD48^-CD41^-$ HSCs, suggesting that the CAR population contained niche cells (111). Other groups evaluating the mechanisms of HSC migration out of the BM revealed the unexpected role for nerve fibers of the SNS in enforced G-CSF-induced mobilization (107). Studies have also shown that, under steady state, adrenergic signals from the SNS delivered by the β3 receptor expressed by the stromal cell mediate circadian oscillations of HSC egress in both mice (99) and humans (112). In addition, adrenergic signals were shown to act on the human $CD34^+$ cells directly by promoting their proliferation and migration (113). Because local nerves were essential to entrain circadian rhythms in the BM, it became apparent that following the innervation pattern might provide some insight about the nature of the targeted niche cell. Sympathetic nerves are intimately associated with the blood vessels that irrigate the marrow (114), where they have been suggested to form a neuroreticular complex (115), and this fact suggested that the niche cell was associated with the vasculature. In addition, the β3 receptor was expressed on osteoblast precursors but not on more committed osteoblast lineage cells (99), indicating that the MSPC might be the cell targeted by the SNS.

Using mice expressing GFP under the Nestin promoter, investigators showed that a rare population of BM $Nestin^+$ perivascular cells was innervated by SNS fibers and expressed relatively high levels of β3 adrenergic receptor and CXCL12 (31). $Nestin^+$ cells also expressed high levels of other HSC regulatory molecules including Angiopoietin-1, SCF, and VCAM-1. In addition, the expression of these molecules was downregulated by signals that trigger HSC release such as G-CSF- and β-adrenergic-induced mobilization (31). $Nestin^+$ cells met other criteria defining a niche cell, including (*a*) physical proximity to most HSCs; (*b*) the generation of ectopic bone and BM with the donor-derived stroma and host-derived HSCs upon transplantation of clonal mesenspheres; and (*c*) rapid HSC loss from the BM upon depletion of $Nestin^+$ cells by diphtheria toxin injection in $Nes\text{-}Cre^{ERT2}$; iDTR mice (31). Although mouse MSCs have not been reported to express CD146, further studies are needed to determine whether $Nestin^+$ cells are the mouse equivalent of human $CD45^-CD146^+$ mural cells that were also shown to reconstitute hematopoietic activity in heterotopic transplantations and to express high levels of HSC regulatory genes (*CXCL12*, *ANGPT1*, and *JAG1*) (32). In a follow-up study, the Nagasawa laboratory (101) generated mice in which a diphtheria toxin receptor–GFP fusion protein was placed under the control of the *Cxcl12* locus. Injection of diphtheria toxin depleted CAR cells and led to HSC loss in the BM, indicating that the CAR cell population contained cells with niche activity. In addition, they showed that these cells produced most of the SCF and CXCL12 in the BM, exhibited multipotent progenitor activity, and were able to differentiate in vivo into osteoblasts and adipocytes (101). Taken as a whole, these studies strongly indicate that MSPCs form the HSC niche.

The nature of the niche was further defined and rendered more complex by recent data from the Morrison laboratory (103) in which the cell origin for SCF synthesis was evaluated. We have known for years that SCF is produced in nontransplantable BM stromal cells. Morrison and colleagues (103) found that, when it was under the control of the *Scf* locus, GFP

expression mostly localized around sinusoids. The authors made tissue-restricted deletions of *Scf* or knock-in expression of GFP in osteoblast, perivascular, or endothelial cells. In particular, they used Cre-mediated deletion of *Scf* driven by the Leptin receptor (LepR-*Cre*) in perivascular cells, which altered HSC numbers in the BM. Deletion in endothelial cells via Tie2-*Cre* also reduced HSC activity in the BM, suggesting that both perivascular and endothelial cells contribute to SCF synthesis in the BM. Unexpectedly, the authors did not observe a contribution from Nestin$^+$ cells using Nes-*Cre* reporter mice, which suggested that LepR$^+$ and Nestin$^+$ cells are distinct. However, Nestin$^+$ cells appear to express high levels of both *Scf* and *Lepr* (31), and LepR$^+$ cells express high levels of CXCL12 (103); some overlap may thus occur among CAR, Nestin$^+$, and LepR$^+$ cells that requires further investigation (**Figure 4*b***).

Several studies showed that cultured endothelial cells promote the expansion of human and murine hematopoietic progenitors (116, 117) and can maintain HSC function in vitro (117, 118). In addition, transplantation of endothelial cells increases HSC radioprotection and accelerates BM recovery after total body irradiation (119). The reasons for this are unclear. Although radioprotection may be due to increased HSC-supportive function by the donor endothelial cells, it could also be due to increased vascular regeneration, which precedes BM regeneration (120). Gain-of-function studies in endothelial cells transduced with a constitutively active form of Akt (*myrAkt1*) have suggested that endothelial cells can support HSC expansion in culture through a direct cell contact mechanism (121). Conditional expression of *myrAkt1* specifically in endothelial cells led to a tenfold increase in HSC frequency in the BM, suggesting that endothelial cells directly promote HSC function (121). Strong evidence supporting a role for endothelial cells in HSC maintenance also comes from specific gene manipulation using Tie2-*Cre* mice to delete the *Gp130* gene in both hematopoietic and endothelial cells (122). Gp130 is a signaling subunit shared by receptors of the IL-6 family of cytokines (122). Tie2-*Cre*; *Gp130$^{fl/fl}$* mice showed progressive BM failure and reduced HSC numbers in the BM. The authors concluded that this was not due to a cell-autonomous defect, given that transplantation of wild-type HSCs into Tie2-*Cre*; *Gp130$^{fl/fl}$* recipients failed to rescue hematopoiesis (122). These data, combined with the aforementioned study of conditional *Scf* deletion (103), indicate that endothelial cells participate in the HSC niche. If this were the case, one would predict the existence of endothelial subsets endowed with niche activity. Although distinct endothelial microdomains have been described (120, 123), more work is needed to define these subsets.

Nerves from the SNS run along the vasculature. Recent data from the Nakauchi laboratory (124) suggest that perivascular Schwann cells that protect sympathetic nerves are themselves a component of the HSC niche. Schwann cells, marked by glial fibrillary acidic protein (GFAP), express β8-integrin, an adhesion molecule thought to activate transforming growth factor-β1 (TGF-β1). TGF-β1 is a powerful cytokine capable of inducing HSC quiescence through the inhibition of lipid raft clustering and the assembly of growth factor signaling microdomains (125). GFAP$^+$ BM cells were found in close proximity to ∼23% of CD150$^+$CD48$^-$CD41$^-$Lin$^-$ HSCs, a frequency lower than that described for CAR cells (97%; Reference 111) or Nestin$^+$ MSCs (60%; Reference 31). In addition, although Schwann cells isolated from the sciatic nerve expressed major niche factors (CXCL12, SCF, Angiopoietin-1, and Thrombopoietin), GFAP$^+$ cells could not be extracted from the BM. Functional data were obtained by transection of the postganglionic sympathetic nerve, which led to the loss of GFAP$^+$ BM cells, increased HSC proliferation, and reduced HSC frequency (124).

NICHE ACCESSORY CELLS

Primary stromal cultures enriched in adipocytes were shown decades ago not to support

HSCs (126), suggesting that adipocytes may negatively regulate hematopoiesis. Subsequent studies revealed that adipocytes secreted adiponectin, which impaired the proliferation of hematopoietic progenitors (127). More recently, studies from the Daley laboratory (128) showed that adipocyte-rich murine bones (tail vertebrae) have reduced HSC frequency when compared with adipocyte-poor bones. In mice that cannot form adipocytes owing to the expression of a dominant-negative form of C/EBP under the adipocyte fatty acid–binding protein 4 promoter (A-ZIP/F1 mice), the tail vertebrae showed normal HSC frequency, supporting the notion that adipocytes are negative regulators of HSC function (128). The question of whether the niche itself mediates this effect remains unanswered. In a mouse model of BM regeneration, the postinjury phase was accompanied by increased osteogenesis (129). Treatment of mice with a peroxisome proliferator–activated receptor γ antagonist inhibiting adipocyte differentiation (130) increased osteogenesis and accelerated BM recovery (128). Because MSPCs can contribute to osteogenesis and adipocyte formation (31, 101), the balance between these two processes likely regulates niche activity.

Osteoclasts have been implicated in the regulation of HSC migration, but their roles are controversial. The fact that calcium-sensing receptor expression was required to direct HSC homing to endosteal marrow suggested that regions with increased calcium availability might surround the HSC niche (131). Osteoclasts may promote HSC mobilization from the BM by the release of cathepsin K that cleaves CXCL12 and thus elicits HSC egress in the circulation (132). However, acute treatment of mice with a bisphosphonate (zoledronic acid, ZA), a family of drugs that inhibit osteoclast function, did not impact HSC mobilization, suggesting that osteoclasts do not directly regulate the HSC niche during G-CSF mobilization (133). In mice chronically treated with ZA, bone mass was increased and HSC numbers were reduced (134). Additionally, mice treated with a different bisphosphonate (alendronate) showed impaired HSC engraftment after transplantation of wild-type HSCs, suggesting reduced niche function after osteoclast inhibition (134). Osteopetrotic mice, including RANKL-deficient animals lacking osteoclasts, reportedly do not show reductions in HSC mobilization (135). Other studies have revealed that in mice subjected to chronic ZA administration or in mice that lack osteoclast activity from a mutation in the *Tcirg1* gene (*oc/oc* mice), the number of HSCs and the size of the BM cavity are reduced; this was accompanied by a proportional increase in phenotypically immature (PDGFRα^+) mesenchymal cells with a reduction in the expression of niche genes (*Angpt1*, *Jag1*, *Cxcl12*, and *Scf*) and osteoblast commitment (136). The changes in mesenchymal cells (increased proportion of immature cells and reduction of osteoblasts) have prompted the authors to suggest that osteoblast differentiation was required for niche formation. However, the reduction in cellularity in this model is such that all mesenchymal cells are likely to be reduced in numbers and altered in function. Thus, osteoclastic activity appears to alter mesenchymal niche-forming cells in the BM, but the specific players involved in this cross talk remain to be elucidated.

Studies using radiation chimeras from mice lacking the G-CSF receptor (encoded by the *Csf3r* gene) revealed that expression of the receptor on a transplantable hematopoietic cell was required for optimal HSC mobilization (137). Several publications have suggested that protease activity derived from myeloid cells cleaved CXCL12 and other molecules in the BM microenvironment (138–140). Recent investigations to identify these cells have led to three independent reports suggesting that BM macrophages act on niche cells to regulate HSC trafficking (133, 141, 142).

In one study (133), G-CSF treatment reduced the number of BM macrophages associated with the bone surface, referred to as osteomacs, and hypothesized that this phenomenon might play a role during mobilization. After depletion of mononuclear phagocytes, bone osteoblast function was suppressed, and this was associated with reduced levels of

CXCL12, SCF, and Angiopoietin-1, suggesting that BM phagocytes can modulate the niche. Another study used transgenic mice in which the *Csf3r* gene was expressed under the control of the *Cd68* promoter, which directs expression to BM monocytes/macrophages (141). These mice were then bred onto a *Csf3r*$^{-/-}$ background, and thus the G-CSF receptor was exclusively expressed by monocytes/macrophages in these mice. G-CSF injection induced normal levels of HSC mobilization, suggesting that Csf3r expression on monocytes/macrophages was sufficient for mobilization (141). An independent set of studies demonstrated that BM macrophages are defined by the CD115^{+}Gr-1^{-}F4/80^{+}CD169^{+} phenotype, where CD169 is restricted to macrophages (i.e., it is not expressed on monocytes) (142). Using mice in which the diphtheria toxin receptor was under the control of the *Cd169* locus, Chow et al. (142) found that macrophage depletion led to HSC mobilization into the circulation, mediated by the downregulation of HSC retention molecules (*Cxcl12*, *Scf*, *Vcam1*, and *Angpt1*) in Nestin^{+} MSCs, suggesting direct cross talk between macrophages and MSCs. These three reports (133, 141, 142) thus demonstrate a specific role of BM macrophages in regulating niche function to direct HSC trafficking.

Finally, recent observations revealed that regulatory T cells (Tregs) endowed the HSC niche with immune privilege. Intravital microscopy imaging studies have shown that after transplantation of fluorescently labeled HSCs in an allogeneic setting, HSCs can survive in the host BM for long periods. Their survival is ensured by the accumulation of CD4^{+}CD25^{+}Foxp3^{+} Tregs, given that depletion of Foxp3^{+} cells leads to rapid loss of allogeneic HSCs. This indicates that Tregs are providing immune protection to the HSC niche (143).

IMMUNE MODULATION BY MSPCs

There has been great excitement about cell therapy using MSPCs because human stromal cells can be easily grown. Initial studies had revealed that MSPCs inhibited T cell proliferation in vitro (144) and were immunosuppressive in a skin allograft rejection model (145), indicating their potent anti-inflammatory properties. In recent years, these effects have been examined in much greater detail, initiating clinical trials with promising results (reviewed in 9, 10 and discussed below). Surprisingly little, however, is known about the precise identity of the stromal cell population(s) that exerts these immunomodulatory functions.

Cellular Origin of Immune Regulation

Stromal cells in various tissues (including dermal fibroblasts) show many of the properties associated with MSPCs in that they adhere to plastic and express similar surface markers as those suggested to identify multipotent mesenchymal stromal cells (20), including CD73, CD90, and CD105. Fibroblasts may also exhibit tri-lineage differentiation potential (146) and anti-inflammatory properties (reviewed in 11). Other studies, however, show that MSPCs are more immunosuppressive than fibroblasts in vivo (147). Still, their gene expression profiles are remarkably similar (148). A confounding problem is the purity of the stromal cell populations because most studies have used commercial sources that employed different harvesting procedures. Thus, these differences could have arisen from contaminating pericytes because the populations tested in these studies were nonclonal. It is therefore difficult at present to attribute unique functions to a particular stromal cell type. Great care should thus be taken when translating findings obtained from studies investigating immunomodulatory functions in preclinical models to the bedside (14).

Immune Targets

Although the precise mechanisms by which MSPCs exert their functions are still unclear, their immunosuppressive capacity is generally accepted. This effect appears to be largely due to suppression of T cell proliferation, which

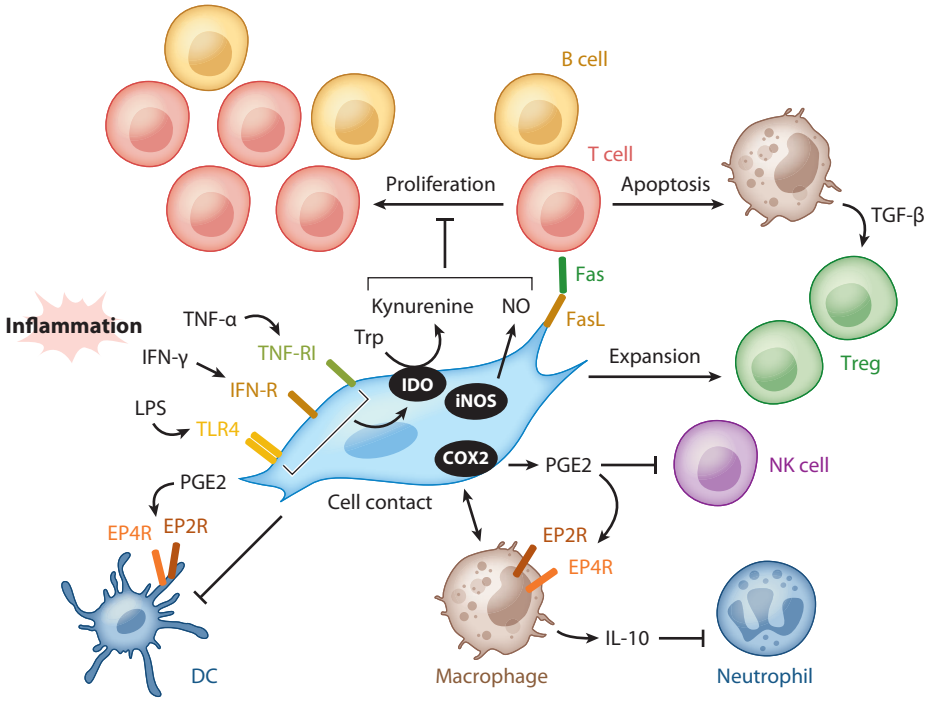

Figure 5

Immune modulation by MSPCs. MSPCs exert immunomodulatory functions in an inflamed environment where IFN-γ and other proinflammatory cytokines such as TNF-α as well as lipopolysaccharides (LPS) can act on their cognate receptors expressed by MSPCs. In human MSPCs, the ensuing intracellular signaling events induce the upregulation of indoleamine 2,3-dioxygenase (IDO) and the production of the tryptophan metabolite kynurenine, which suppresses the proliferation of T and B lymphocytes. In murine MSPCs, cytokine-induced signaling promotes the expression of inducible nitric oxide synthase (iNOS), which produces similar antiproliferative effects on T and B cells. MSPCs can indirectly induce the expansion of regulatory T cells (Tregs) in vivo, most likely via TGF-β released by macrophages, which is triggered by FAS-ligand-/FAS-mediated apoptosis of T cells. Anti-inflammatory properties of MSPCs on dendritic cells (DCs) and macrophages are mediated via the enzyme cyclooxygenase 2 (COX2) and its effector molecule prostaglandin E2 (PGE2). PGE2 binds to E prostanoid receptors (EP2R and EP4R) on DCs and macrophages, which induces a tolerogenic state in DCs and promotes the release of IL-10 by macrophages, inhibiting neutrophil infiltration to tissues. Released PGE2 is also a potent inhibitor of NK cell function.

may also explain the antiproliferative effect on B cells (149, 150). However, MSPCs can also affect other immune cells, including natural killer (NK) cells, dendritic cells (DCs), and macrophages (**Figure 5**).

T cells. MSPCs have been shown repeatedly to suppress mitogen- or alloantigen-driven T cell proliferation, but they appear to have little impact on virally induced responses (151). The antiproliferative properties of MSPCs on T cells can be reversed via addition of IL-2 (144, 145). In addition to CD4 T cells, MSPCs can inhibit the proliferation of CD8 T cells in cytolytic and alloreactive responses but have no effect once these cells have become activated (152, 153). MSPCs also inhibit the proliferative response of unconventional T cells such as invariant NKT cells and γδ T cells (154). Importantly, coculture of MSPCs with T cells

favors the generation of Tregs (155), and in vivo expansion of Tregs was also observed after infusion of MSPCs (156). Induction of Treg numbers was triggered by release of TGF-β by macrophages, which was dependent on interactions between FAS-ligand expressed by MSPCs and FAS on T cells, leading to T cell apoptosis (157, 158).

Natural killer cells. Because of their relatively low expression of major histocompatibility (MHC) class I, MSPCs are not inherently immunogenic (159). In addition, they appear to be able to elude NK-mediated lysis by inhibiting cytokine-induced proliferation of NK cells and blocking the induction of effector functions via release of prostaglandin E2 (PGE2) (159, 160). In a manner similar to cytolytic CD8 T cells, however, once activated, NK cells can lyse MSPCs efficiently (159).

Dendritic cells. MSPCs have been reported to induce a tolerogenic, immature state in DCs, which is characterized by a decrease in the expression of MHC class II and the costimulatory molecules CD40, CD80, and CD86 as well as by reduced secretion of IL-12. This indirectly leads to the suppression of T cell proliferation via a DC-mediated pathway (161, 162). A major mechanism appears to be mediated by MSPC-derived PGE2 acting on the E prostanoid receptors EP2R and EP4R expressed by DCs (163, 164).

Macrophages. In an experimental sepsis model, MSPCs were shown to program host macrophages in a TLR4-mediated manner by releasing PGE2, which like DCs acts on EP2R and EP4R expressed by macrophages. Signaling through E prostanoid receptors leads to increased secretion of the anti-inflammatory cytokine IL-10 by macrophages, preventing neutrophils from migrating into tissues and thus averting multi-organ damage (147).

MOLECULAR BASIS OF MSPC-INDUCED IMMUNOSUPPRESSION

As suggested by recent studies (147, 165), the immunosuppressive activity of MSPCs may require prior activation with proinflammatory factors such as IFN-γ and either TNF-α, IL-1α, or IL-1β, highlighting the importance of an inflammatory milieu. Among these cytokines, IFN-γ appears to be the most important, and IFN-γ receptor expression by MSPCs has also been implicated (147, 165). However, human MSPCs that lack IFN-γ receptor expression can still exhibit immunosuppressive effects (166), and high levels of IFN-γ and TNF-α have been shown to synergistically induce MSPC apoptosis (156).

There is contention regarding the mechanism by which MSPCs exert their effects once they are activated: Is direct contact with target cells needed, or are soluble factors sufficient? Close proximity is clearly necessary to promote immunomodulation, and direct contact can further enhance the beneficial effect (14). Expression of many adhesion molecules—such as CD90, CD106, and various integrins (167)—on the cell surface would support direct interactions with the local environment and immune cells. Initial release of soluble chemokines by MSPCs such as CXCL9, CXCL10, and CXCL11 appears to be important for the stromal cell to reach the immune cell (165, 168), allowing soluble factors with a limited diffusion range to mediate immunosuppression. In addition, recent studies in murine models of sepsis showed the requirement of direct interactions between MSPCs and macrophages (147), and other elegant analyses have suggested the transfer of mitochondria between MSPCs and lung epithelium to alleviate inflammation (169). For the latter, gap junctions expressing Connexin 43, a protein prominently expressed in murine MSCs (31), were required. These studies suggest the requirement of local infiltration by MSPCs.

Several soluble candidate factors released by MSPCs have been suggested as mediators

of immunosuppression. Featuring prominently among them is nitric oxide (NO). In the presence of IFN-γ and either IL-1α, IL-1β, or TNF-α, MSPCs upregulate the expression of inducible NO synthase (iNOS), leading to NO secretion, which suppresses T cell proliferation (165, 170). MSPCs from mice that lack iNOS showed a reduced suppressive capability both in vitro and in vivo (165). Expression of iNOS in MSPCs appears to be dependent on the transcription factor C/EBPβ (171). NO may then in turn inhibit phosphorylation of Stat5 in T cells, antagonizing proliferation (170). Although strong experimental evidence exists for this IFN-γ/NO axis in mice, human MSPCs express lower iNOS levels and do not suppress T cell proliferation via NO (168). Instead, the enzyme indoleamine 2,3-dioxygenase (IDO) has been proposed as a key effector for human MSPCs. IFN-γ stimulation upregulates IDO production, which converts the essential amino acid tryptophan into its metabolite kynurenine, exerting antiproliferative and thus anti-inflammatory effects (168, 172). Additionally, cyclooxygenase 2 and its effector molecule PGE2 inhibit T cell mitogenesis and IL-2 production (173, 174). Other soluble factors implicated in MSPC-mediated immune regulation include TGF-β1, hepatocyte growth factor, heme oxygenase-1, IL-6, leukemia inhibitory factor, human leukocyte antigen G5, and IL-10, all of which can act as potential effectors of MSPC-mediated immunosuppression (reviewed in 12). Inhibition of any one of these factors, however, results in only a partial reduction of the immunosuppressive effect of MSPCs; this suggests that several independent mechanisms are involved.

MSPCs IN CANCER

Given their dual functions as regulators of the immune response and enablers of stem cell niches, MSPCs sit at the nexus of cancer immunology and cancer stem cell biology, both of which likely have profound influence on cancer progression. MSPCs reportedly enter tumors via chemokine secretion by cancer cells (175, 176). The recruitment of MSPCs to tumors has led to the idea that they could serve as delivery vehicles for antitumor biological modifiers, a notion supported with preclinical studies using IFN-β-, IL-2-, or TRAIL (TNF-related apoptosis-inducing ligand)-expressing MSPCs in melanoma or glioma models (177–180). Human MSPCs may alter the behavior of cancer cells upon exposure to tumor-conditioned medium by differentiating into fibroblast-like cells that can promote tumor cell growth (181). Other studies have suggested that MSPCs could enhance cancer metastasis when close to the engrafted breast cancer cells through the release of CCL5, which binds to its receptor CCR5 on the cancer cells (182). One study (183) suggested that secretion of a disintegrin and metalloprotease 10 (ADAM10) by MSPCs enhances breast cancer cell migration through the cleavage of E-cadherin. This effect may not be apparent in advanced tumors that have already lost E-cadherin expression (182). In contrast, MSPCs have also been linked with inhibiting tumor growth of rat colon carcinoma cells (184) or in a model of Kaposi's sarcoma (185). MSPCs have multiple effects on the tumor microenvironment (e.g., vascular support, fibroblast network, recruitment of immune cells, secretion of a myriad of soluble factors) that could affect cancer development and metastasis either positively or negatively (reviewed in 13). It is likely that the heterogeneity of MSPC populations—which is due to the various tissue and developmental sources of MSPCs and to the in vitro expansion of the cells—contributed to the differences in responses. These data emphasize the need to carry out such studies with more homogeneous cell populations.

THERAPEUTIC IMPLICATIONS OF MSPCs

Pioneering studies defining the multilineage potential of cultured MSPCs have set the stage for multiple therapeutic applications (1, 5, 19). MSPCs have been at the center of emerging clinical investigations for a surprising variety of conditions encompassing immunomodulation,

regenerative medicine, tissue protection, and graft enhancement (**Table 1**) (reviewed in 9, 10, 14). Many of the ongoing trials are testing the potential beneficial effects of MSPCs for mitigating graft-versus-host disease (GvHD), multiple sclerosis, type 1 diabetes, and Crohn's disease (**Table 1**). Keen interest is also directed at assessing the ability of MSPCs to protect tissue following injury. Significant effort has been invested in the setting of myocardial infarction where several preclinical studies have suggested that MSPC infusion can lead to reductions in infarct size and improve contractile function in myocardial infarction models (186). However, the clinical benefits in various pilot stem cell therapeutic trials have been relatively modest (187). As is the case for all types of cell therapy, there remain significant obstacles to bringing it to the clinic, including the risk of immune rejection of allogeneic or xenogeneic cells, risk of pathogen contamination, high costs, effectiveness of cell delivery, and the in vivo behavior and safety of the long-term transplanted cells.

Hurdles for Cell Therapy

Cultured MSPCs have been infused clinically, mostly for their regenerative purposes but also for their anti-inflammatory properties. Although the infusion of allogeneic MSPCs would be expected to induce an immune response, they have generally been considered to be immune privileged. BM-derived stromal cells express low levels of MHC class I but lack expression of MHC class II and the costimulatory molecules B7 and CD40 ligand (188). However, upregulation of both MHC class I and class II on MSPCs has been observed after stimulation with IFN-γ (189, 190), lending them the capacity to present antigen and induce effector T cell responses in vitro and memory T cell responses in vivo (191, 192). Nonetheless, data obtained from preclinical animal models and clinical trials using both syngeneic and allogeneic MSPCs have thus far not elicited major adverse events (193, 194). Donor MSPCs can persist in recipients for months, although reports exist of T cell–mediated apoptosis (156, 159). However, a study has linked administration of MSPCs without additional immunosuppressive therapy with the acceleration of graft rejection in rats (195). In addition, malignant transformations of injected human MSPCs in mouse models of acute myocardial infarction and diabetic neuropathy have been reported (196). Such undesirable outcomes highlight the need for a better overall understanding of injected MSPCs and their effects so that potential therapy can be tailored effectively to patients.

The route of administration represents a critical variable for therapeutic delivery, as do the mechanisms by which infused cells reach the target site (reviewed in 15). Although intravenous injection is the most common and least invasive route of administration, intra-arterial injection may achieve better results because it decreases the risk of passive entrapment in the lung. In some cases, local injection of MSPCs has been used as a site-specific delivery method. Currently, no definitive in vivo homing mechanism exists for MSPCs, making it difficult to decipher whether infused cells have engrafted into tissues and differentiated to replace injured cells or whether they are still localized within the vasculature (15). MSPCs tend to integrate closely to the endothelial layer, in a manner similar to pericytes. Engraftment of donor MSPCs within tissues may also result from fusion with endogenous cells (45). In addition, MSPCs passively arrest in small diameter vessels such as capillaries, small arterioles, and postcapillary venules (197, 198). However, active mechanisms have been described, using parallel plate flow chambers, demonstrating that the interactions between the endothelium and the MSPCs were mediated by P-selectin and VCAM-1, the latter through interactions with the integrin very late antigen-4 (199, 200). Because MSPCs express relatively low levels of homing molecules such as selectin ligands, one group (197) carried out in vitro engineering of E-selectin ligands using recombinant fucosyltransferase VI to enhance MSPC migration in the BM following intravenous injection. A similar approach was used to enhance homing of cord blood–derived HSCs (201, 202), which is currently being evaluated

in a clinical trial. It is believed that in response to tissue injury, MSPCs can home to the site of damage and encourage repair through the production of trophic factors, including growth factors, cytokines, and antioxidants (15), some of which provide the basis for their capacity to modulate immune responses. Nevertheless, the overall engraftment rate of MSPCs into injured tissues is low compared with the functional recovery observed after transplantation, suggesting that paracrine factors and not local engraftment and differentiation account for the beneficial effects.

Regenerative Medicine from Within

The ability to grow bone or cartilage precursors has spurred applied research in cartilage regeneration in the context of osteoarthritis, the most common joint disease in adults. Local administration of cell preparations in animal models and pilot clinical trials have been encouraging (203–205). Recent promising studies have revealed the regenerative potential of recruiting reprogrammed endogenous cells. Using an anatomically designed polycaprolactone and hydroxyapatite bioscaffold loaded with TGF-β3 in a rabbit articular cartilage model, endogenous cells were recruited to the site and able to regenerate the entire surface of the synovial joint (206, 207). In a large-defect sheep bone model, composite polycaprolactone and tricalcium phosphate scaffolds combined with recombinant human BMP-7 were recently shown to regenerate bone more efficiently than autologous cancellous bone grafts or scaffolds combined with autologous BM-derived mesenchymal stromal cells (208). Another approach that could be combined with the current homing and transplantation studies is the use of bioactive molecules such as LLP2A-Alendronate, which induces the migration of endogenous MSPCs toward the bone and stimulates osteoblastic differentiation augmenting bone formation and bone mass (209). One recent study showed that the intra-articular administration of kartogenin was able to direct the differentiation of cartilage-resident MSPCs, leading to in vivo improvements in damaged joint models (210).

Building an Artificial Niche for HSC Expansion

Although the transplantation of HSCs is by far the most common stem cell therapy in use today, many individuals who need transplantation do not have a suitable allogeneic donor. In these cases, unrelated cord blood–derived HSCs have shown promise given the ease of collection, cryopreservation potential, and relatively low potential to elicit GvHD compared with BM-derived adult cells (reviewed in 18). The main limitation of cord blood–derived cells as a source of cells for transplantation is that the number of HSCs recovered in a single cord is generally too low for transplantation into adults. In these cases, infusion of two separate cords has been used with increasing frequency but can still lead to delayed hematopoietic recoveries (211). To improve the clinical outcome of HSC transplantation, many groups have focused on the ex vivo expansion of HSCs and progenitors, particularly for cases such as cord blood units in which the graft size is a limiting factor (212).

Various strategies have been used to expand cord blood–derived HSCs in vitro, including treatments with angiopoietin-like proteins, insulin growth factor–binding protein 2 (213, 214), the Notch ligand Delta 1 (215), the purine derivate StemRegenin1 (an antagonist of the aryl hydrocarbon receptor) (216), and dimethyl-PGE2 (217–219). However, because ex vivo liquid culture removes HSCs from critical molecular cues provided by the hematopoietic microenvironment, the addition of exogenous cytokines is required in most of the cases to prevent apoptosis and to stimulate proliferation (220). An alternative approach is the ex vivo coculture of HSCs with aforementioned components of their hematopoietic microenvironment.

BM mesenchymal stromal cells have long been proposed as sources of regulatory signals to hematopoietic progenitors, given that mixed

cultures derived from the adherent fraction of the BM promote the maintenance of HSCs in vitro (221). During the past two decades, numerous studies have explored the ability of mesenchymal stromal cultures to support the ex vivo expansion of HSCs (222–230). In most cases, the addition of hematopoietic cytokines to cultures or genetic alteration of MSPCs was required to maintain hematopoiesis. However, these systems were able only to expand hematopoietic progenitors and failed to preserve primitive HSCs with long-term multilineage engraftment capacity (18). An important consideration is the fact that the populations of mesenchymal stromal cells are highly heterogeneous, and further studies should be designed using purified populations of MSPC candidates to test their capacity for expansion of engraftable long-term HSCs. Because multiple cell types contribute to the niche, additional studies combining niche constituents may improve the yield, although such complex multicellular systems would likely represent a great challenge for clinical translation (231).

PERSPECTIVE

It is humbling to look back at the vision, stated a quarter century ago (65), that the stromal cells in the BM were likely organized hierarchically in a manner as complex as their hematopoietic counterparts. Although much progress has been made in defining lineage-committed hematopoietic progenitors, our knowledge remains limited about the stromal cells that orchestrate the complex balance between supply and demand that regulates hematopoietic cell differentiation and their release in the circulation.

Despite this knowledge gap, MSPC-based therapies have rapidly ascended among the most commonly used and studied stem cell treatments of US-registered clinical trials (**Table 1**; see **http://www.clinicaltrials.gov**). Although they hold great promise, there is currently no Food and Drug Administration (FDA)-approved indication for MSC infusion, and thus such therapies must remain under the purview of carefully controlled clinical trials. There is concern among the scientific community that the explosion of makeshift stem cell clinics, selling services for unproven conditions, may ultimately cause irreparable damage to a promising field. Although most of the political discussions have revolved around cell therapy using embryonic stem cell–derived products, MSCs have been at the forefront of adult stem cell alternatives for a range of conditions (232). In the patient handbook on stem cell therapies published by the International Society for Stem Cell Research (**http://www.isscr.org**), major warning signs of dubious stem cell therapies are claims that multiple diseases can be treated by the same cells. Unfortunately, MSPC-based treatments currently fit this description. This stresses the need to precisely define the cell types that form what the FDA has now labeled an orphan drug and identify its active ingredients. Identification of active molecular players would lead to the development of classical drugs that target the pathways activated by MSPCs. Therefore, defining whether the activities are derived from genuine stem cells or other stromal components is not an academic exercise but rather the only way to ensure sustained, long-term progress in cell-based therapeutics.

DISCLOSURE STATEMENT

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