Bone Marrow Mesenchymal Stem Cells

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ABSTRACT

Following the identification of bone marrow multipotent cells that could adhere to plastic and differentiate along numerous mesenchymal lineages in vitro, a considerable effort has been invested in characterizing and expanding these cells, which are now called "mesenchymal stem cells" (MSCs), in vitro. Over the years, numerous lines of evidence have been provided in support of their plasticity, their extraordinary immunomodulatory properties, their potential use for tissue engineering purposes, as well as their ability to be recruited to sites of injury, where they might contribute a "natural in vivo system for tissue repair." Moreover, some studies have attempted the characterization of their cell-surface specific antigens and of their anatomical location in vivo. Lastly, it has been shown that similar cells could be also isolated from organs other than the bone marrow. Despite this impressive body of investigations, numerous questions related to the developmental origin of these cells, their proposed pluripotency, and their role in bone modeling and remodeling and tissue repair in vivo are still largely unanswered. In addition, both a systematic phenotypic in vivo characterization of the MSC population and the development of a reproducible and faithful in vivo assay that would test the ability of MSCs to self-renew, proliferate, and differentiate in vivo are just beginning. This brief review summarizes the current knowledge in the field of study of MSCs and the outstanding questions. J. Cell. Biochem. 109: 277–282, 2010. © 2009 Wiley-Liss, Inc.

The presence of non-hematopoietic stem cells (HSCs) in the bone marrow was first suggested by the German pathologist Cohneim about 130 years ago, who proposed that bone marrow can be the source of fibroblasts contributing to wound healing in numerous peripheral tissues [Schipani and Kronenberg, 2009]. In the 1970s the work of Friedenstein et al. [1970] demonstrated that the rodent bone marrow had fibroblastoid cells that could form colonies on plastic in vitro and were thus named colony-forming unit fibroblasts (CFU-Fs). Notably, in subcutaneous transplants, these cells were able to both form bone and reconstitute a hematopoietic microenvironment [Friedenstein et al., 1974]. Castro-Malaspina et al. [1980] succeeded in isolating CFU-Fs also from human bone marrow. It was then shown that these cells could be subpassaged and differentiated in vitro into a variety of mesenchymal lineages such as osteoblasts, chondrocytes, and adipocytes [Caplan, 2007; Bianco et al., 2008]. Moreover, Pittenger et al. [1999] unequivocally demonstrated that single MSC clones were indeed multipotent as they could give origin to multiple mesenchymal lineages in vitro. Friedenstein had thus isolated from the bone marrow what later Caplan would have renamed "mesenchymal stem cell" (MSC) [Caplan, 1991].

In the last several years, it has been proposed that MSCs could serve as a powerful "natural system for tissue repair," and they could be effective therapeutic agents in a variety of experimental models of tissue injuries [Brooke et al., 2007; Caplan, 2007; Phinney and Prockop, 2007; Abdallah and Kassem, 2008] (see below). A significant number of investigations have thus focused on expanding and phenotypically characterizing MSCs in vitro, in order to then transplant them back in vivo for the purpose of repairing specific tissues including bone and cartilage.

All in all, our knowledge of MSCs is virtually based on studies of cultured cells. Conversely, the phenotypic characteristics of MSCs in vivo are still largely unknown. Moreover, a faithful assay that would prove their "true stemness" in vivo by testing for their ability to self-renew in vivo is not yet available. Lastly, in vivo clear and solid evidence that MSCs are indeed the skeletal stem cells is still missing, despite a variety of very elegant studies that correlate the number of CFU-Fs to bone mass [Bonvadi et al., 2003; Hilton et al., 2008].

This brief review summarizes the current knowledge in the field of study of MSCs and the outstanding questions.

ISOLATION AND IN VITRO CHARACTERIZATION OF BONE MARROW MSCs

In addition to the bone marrow, MSCs or MSC-like cells have been also found in tissues such as fat, umbilical cord blood, amniotic fluid, placenta, dental pulp, tendons, synovial membrane, and skeletal muscle [Phinney and Prockop, 2007; Schipani and Kronenberg, 2009], though the complete equivalency of these populations has not been formally demonstrated using robust scientific methods.

As described above, the initial isolation method of bone marrow MSCs took advantage of their property, differently from hematopoietic cells, to adhere on plastic. However, only some clones isolated according to Friedenstein's method were osteogenic, which indicated that these cultures were indeed heterogeneous [Kuznetsov et al., 1997]. Numerous studies have thus focused on identifying methods for the isolation of a more homogeneous population of MSCs. In this section, we will briefly summarize the various isolation methods that have been optimized by researchers in the attempt to purify a more homogeneous population of human MSCs (hMSCs).

In this regard, Percoll gradients have been shown to be particularly useful. First developed by Caplan and colleagues [Haynesworth et al., 1992], the method was later modified by Pittenger et al. [1999]. Overall, the hMSC lines established by this group were quite homogeneous, as shown by flow cytometric analysis of specific cell-surface antigens (see below). Culturing bone marrow cells in low oxygen tension has also been demonstrated to considerably increase the homogeneity of MSC cultures [D'Ippolito et al., 2004; Carrancio et al., 2008].

Numerous cell-surface antigens commonly expressed in vitro by hMSCs have been identified, including CD90 (Thy1), CD106, CD29, CD166, CD44, CD73, and CD105 [Chamberlain et al., 2007; Phinney and Prockop, 2007]. Recently, the International Society for Cellular Therapy (ISCT) has announced the criteria to be followed in order to define bone marrow cells as hMSCs. According to these criteria, MSCs are required to be positive for CD73, CD90, and CD105 and with virtually no expression (<2%) of CD45, CD34, CD14, CD19, HLA-DR, in addition to displaying properties such as adherence to plastic and multilineage differentiation potential [Dominici et al., 2006].

IN VIVO MARKERS OF BONE MARROW MSCs

Though numerous cell-surface antigens expressed by MSCs that have been cultured and subpassaged in vitro have been identified over the years, only a few laboratories have attempted a phenotypic characterization of MSCs in vivo, which is still partial and incomplete [Bianco et al., 2008].

In 1980, Simmons and colleagues generated an antibody that would recognize a cell-surface antigen present in human bone marrow stromal cells. The population positive for this antigen, named STRO1, was considerably enriched in clonogenic cells that were able to both generate CFU-Fs and differentiate into multiple mesenchymal lineages in vitro [Simmons and Torok-Storb, 1991]. The same group reported that the degree of homogeneity of the STRO-1-positive population could be further enhanced by positive selection for VCAM/CD106 [Short et al., 2009]. More recently, an important study by Bianco and colleagues has demonstrated that MCAM/CD146(+) cells isolated from the human bone marrow stroma adhere to plastic in vitro and are clonogenic; moreover, they self-renew, at least in vitro, and they can generate bone and a hematopoietic supportive microenvironment in subcutaneous transplants in mice [Sacchetti et al., 2007]. The relationship, if any, between these cells and the STRO-1/VCAM-positive cells previously reported by Simmons' group remains to be established.

Considerably less progress has been made in the characterization of cell-surface antigens expressed by murine MSCs in vivo. Vlasselaer et al. [1994] reported the purification of cells with osteogenic potential from murine bone marrow by two-color cell sorting using anti-Sca1 monoclonal antibody and wheat germ agglutinin. More recently, Simmons' laboratory has identified a bone marrow pool of Sca1(+) CD45(−) CD31(−) cells that appears to be enriched in MSCs/progenitors [Short et al., 2009]. CD45, a pan-hematopoietic cell marker, and CD31 or PECAM, a classical marker for endothelial cells, were used in the study to negatively select for hematopoietic cells and endothelial cells, respectively.

ORIGIN OF BONE MARROW MSCs AND CHARACTERIZATION OF THEIR NICHE

The distinct lineage origin of stromal cells was elegantly demonstrated by Simmons and colleagues, who showed that these cells isolated from patients with functioning sex-mismatched but HLA-identical allografts were exclusively of host genotype [Simmons et al., 1987]. The finding clearly indicated that stromal cells supporting hematopoiesis are a population distinct from hematopoietic cells, and thus confirmed in vivo previous observations from Friedenstein's laboratory, which had shown that in sex-matched transplants cells capable of forming heterotropic osseous tissue were physically different from HSCs [Friedenstein et al., 1974]. Most of the MSC lines established nowadays are indeed negative for the hematopoietic marker CD45.

The current model is that there are at least two types of stem cells in the bone marrow, HSCs and MSCs (Fig. 1). According to this model, HSCs would give rise to hematopoietic cell types and to cells that resorb bone (osteoclasts), whereas MSCs would differentiate into a variety of mesenchymal lineages such as chondrocytes, adipocytes, and osteoblasts.

Recent studies, however, have challenged this clear distinction (Fig. 1). For example, it has been reported that tissue fibroblasts/myofibroblasts, which are thought to play an important role in wound healing, pathological fibrosis, and cancer, may derive from HSCs [Ogawa et al., 2006]. In addition, a study from Olmsted-Davis et al. [2003] has provided evidence that the bone marrow side population, which is apparently enriched in HSCs, could contribute skeletal progenitor cells. Lastly, it has been shown that a subset of CD45(+) Lin(−) bone marrow cells was able to differentiate in vitro
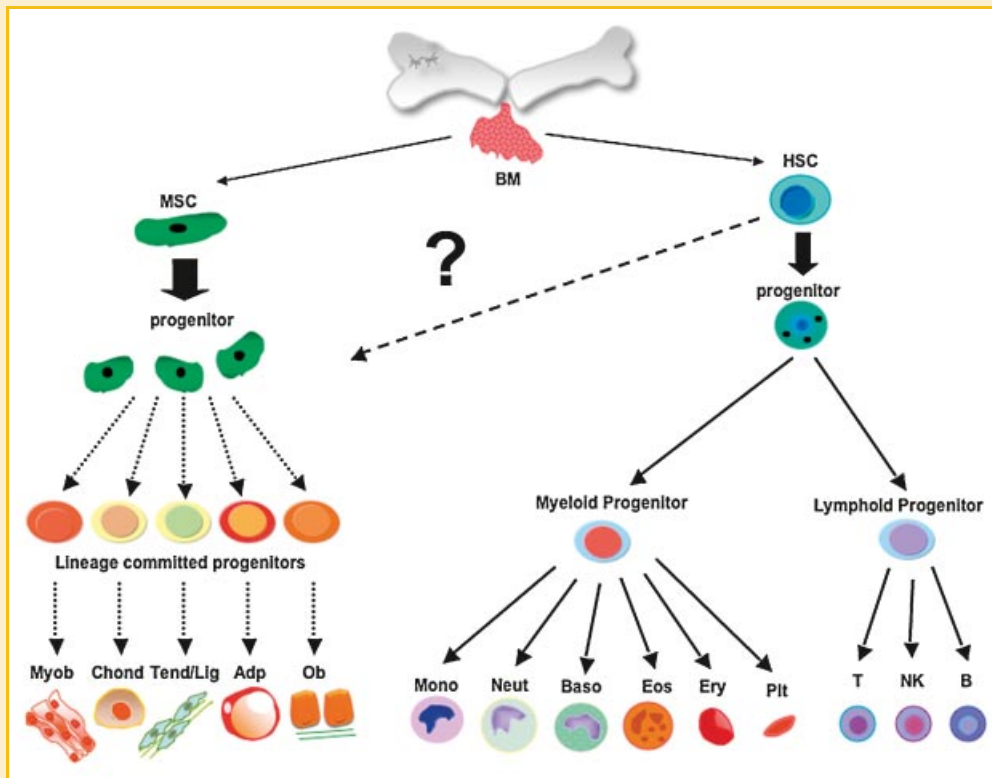
Fig. 1. Mesenchymal stem cells (MSCs) and hematopoietic stem cells (HSCs) in the adult bone marrow. Although osteoblasts (Ob), chondrocytes (Chond), adipocytes (Adp), tendon/ligament fibroblasts (Tend/Lig), and myoblasts (Myob) have been shown to originate from MSCs in vitro, whether one single progenitor exists for each of these mesenchymal lineages in vivo is yet an unanswered question. HSCs differentiate into two different lineage committed progenitors, myeloid progenitors and lymphoid progenitors. Myeloid progenitors differentiate into monocytes (Mono), neutrophils (Neut), basophils (Baso), eosinophils (Eos), erythrocytes (Ery), and platelets (Plt). Lymphoid progenitors differentiate into T lymphocytes (T), B lymphocytes (B), and natural killer cells (NK). Some studies have suggested that hematopoietic cells including HSCs might differentiate into mesenchymal cells lineages. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

into a variety of cell types, including endothelial cells, osteoblasts, muscle cells, and neural cells [Rogers et al., 2007]. Interestingly, others have failed to confirm that MSCs and HSCs may share a common lineage [Koide et al., 2007]. Further investigations are needed to clarify the discrepancies among the different studies, which nonetheless do raise questions about either the reliability of CD45 as an exclusive hematopoietic cell marker or, rather, the existence of a clear boundary between HSCs and MSCs.

Along these lines, it has been recently proposed that pluripotent stem cells exist in the bone marrow, which could give origin to tissues that embryologically derive from all the three embryonic germ layers, ectoderm, mesoderm, and endoderm. This is an appealing and exciting possibility for which, however, only a few pieces of experimental evidence have been provided so far [Jiang et al., 2002; D'Ippolito et al., 2004; Ratajczak et al., 2007].

Even more uncertain is the embryological origin of bone marrow MSCs. It is widely believed that MSCs derive from mesoderm; notably, however, a recent study showed that the earliest lineage providing MSC-like cells during embryonic trunk development is indeed generated from Sox1(+) neuroepithelium rather than from mesoderm, at least in part through a neural crest intermediate stage [Takashima et al., 2007]. These early MSCs are then replaced, later in development, by MSCs from other origins. Consistent with this observation, it has been recently demonstrated that neural crest-derived cells migrate to the bone marrow through the bloodstream [Nagoshi et al., 2008]. These cells are still present in the adult bone marrow and can differentiate in vitro into neurons, glial cells, and myofibroblasts. The potential link, if any, between these cells, the cells identified by Takashima et al. [2007] and the MSCs isolated according to Friedenstein's protocol remains to be established.

In absence of specific and unique markers that would allow for a proper identification of MSCs in vivo, a histological localization of these cells is virtually impossible to identify and is clearly lacking.

An extensive literature has pointed to pericytes as a potential source of MSCs [Bianco et al., 2008; Crisan et al., 2008]. As already mentioned above, Bianco and colleagues have recently reported that MCAM/CD146(+) subendothelial cells in the human bone marrow are the only cell population in the bone marrow that is both clonogenic in vitro and capable of transferring a hematopoietic microenvironment in subcutaneous transplants [Sacchetti et al., 2007]. These cells, which reside in the wall of the sinusoidal blood vessels of the bone marrow, are also positive for angiopoietin-1, a critical regulator of vascular remodeling. The findings by Bianco and colleagues represent the first rigorous attempt to histologically localize and phenotypically define MSC-like cells, or at least a subpool of this population. Notably, a recent paper by Crisan et al.

[2008] suggests that multipotent MSCs with perivascular localization exist in numerous human organs.

Another extremely important but yet unanswered question with regard to MSCs and their site of origin is whether the bone periosteal compartment, which is critical for fracture repair, is also as a source of MSCs and whether this periosteal population shares significant similarities with the MSCs isolated from bone marrow [Zhang et al., 2008].

USE OF BONE MARROW MSCs FOR TISSUE REPAIR AND REGENERATION

As aforementioned, it has been proposed that MSCs could serve as a powerful "natural system for tissue repair" and be effective therapeutic agents in a variety of experimental models of tissue injuries [Brooke et al., 2007; Caplan, 2007; Phinney and Prockop, 2007; Abdallah and Kassem, 2008]. In particular, hMSCs/progenitors have been used, after ex vivo expansion and upon local implantation, to successfully repair bone and cartilage defects, fracture non-unions, chronic skin wounds, and ischemic hearts in humans and in experimental models [Connolly, 1995; Zimmet and Hare, 2005; Vilquin and Rosset, 2006; Abdallah and Kassem, 2008; Bajada et al., 2008]. Direct implantation of hMSCs into the damaged brain has also resulted in functional gain in rats [Zhao et al., 2002].

Systemic administration of hMSCs has lowered glucose levels in diabetic mice [Lee et al., 2006]. Moreover, in animal models, systemically administered bone marrow MSCs have been shown to facilitate repair of damaged kidneys and brain [Chamberlain et al., 2007; McTaggart and Atkinson, 2007]. Systemic administration of bone marrow cells has been attempted in humans to treat osteogenesis imperfecta [Horwitz et al., 2001]. A promising phase 1 placebo-controlled clinical trial in patients shortly after myocardial infarction and upon systemic delivery of allogenic MSCs is in progress. Notably, 6 months after infusion of MSCs, patients improved their cardiac ejection fraction and lung function [Brooke et al., 2007]. Systemic delivery of MSCs has also been performed with some success in patients affected by severe aplastic anemia, and in patients receiving peripheral blood HSC transfusion (PBSCT), the underlying rational being that transplanted MSCs would help with the timely reconstitution of an appropriate niche for HSCs [Brooke et al., 2007].

Despite the variety of attempts to deliver MSCs by systemic administration, a major and severe limitation of this approach is constituted by the very low number of MSCs that home to the site of injury [Marino et al., 2008]. A possible reason for the inefficient engraftment and homing could be the entrapment of MSCs in the lungs [Abdallah and Kassem, 2008].

Moreover, despite the encouraging results of the initial attempts to use MSCs in pre-clinical and clinical settings, the mechanisms responsible for the positive effects secondary to delivery of MSCs to sites of injuries are controversial. Curiously, the therapeutic efficacy of MSCs often did not correlate with their efficiency of engraftment, which, as mentioned above, is in general very low [Phinney and Prockop, 2007]. This observation suggests that the ability of MSCs to repair injuries could be due not to their transdifferentiation into the appropriate cell phenotype or to cell fusion, but rather to the secretion by MSCs of soluble factors that alter the tissue microenvironment. In other terms, MSCs may provide what Caplan and colleagues define as "trophic activity" [Caplan, 2007]. In support of this possibility, extensive proteomic analyses have indeed revealed that MSCs in vitro produce a variety of factors that influence a broad range of biological functions, including angiogenesis, and secrete neuroregulatory peptides and cytokines with critical roles in inflammation and repair [Caplan, 2007].

If the defect to be repaired is such that it would require transplantation of a whole tissue and/or an organ, the use of scaffolds to deliver MSCs could be a more appropriate method in alternative to local implantation or systemic administration. This approach is still at a pre-clinical stage in humans, but studies in animal models with large bone and cartilage defects have shown promising results [Caplan, 1991; Brooke et al., 2007; Abdallah and Kassem, 2008].

OTHER APPLICATIONS OF MSCs

Usage of genetically modified MSCs to deliver specific genes to tissues or organs is another potential application for MSCs. In experimental models, MSCs have been used to express ectopic BMP_2 in order to repair cartilage or bone [Abdallah and Kassem, 2008], or to locally produce anti-inflammatory cytokines in autoimmune disease models [Choi et al., 2008].

Probably the most surprising feature of MSCs is their extraordinary, and still partially unexplained, immunomodulatory properties. Adult hMSCs have been reported to express intermediate levels of major histocompatibility complex (MHC) class I proteins, but not MHC class II proteins. This phenotype has been regarded as non-immunogenic, which implies that hMSC transplant into an allogenic host would not require any immunosuppressive therapy. Even more surprising, numerous studies have shown that MSCs exert their immunosuppressive properties by modulating specific T-cell functions in vitro [Chamberlain et al., 2007]. The anti-inflammatory effect of MSCs could be, at least in part, mediated by secretion of IL-1 receptor antagonist [Phinney and Prockop, 2007].

The immunomodulatory and anti-inflammatory properties of MSCs have led to the usage of these cells as therapeutical tools in in vivo settings such as graft versus host disease (GVHD) and autoimmune diseases [Brooke et al., 2007; Chamberlain et al., 2007; Muller et al., 2008]. Clinical trials for GVHD and Crohn's disease are in progress [Caplan, 2007].

A FUTURE PERSPECTIVE

As described in this summary, MSCs could have enormous potential for clinical use. However, our knowledge of MSCs is almost entirely based on cultured cells. A systematic, phenotypic in vivo characterization of the MSC population would be extremely helpful as it would allow us to learn about the role of these cells in organogenesis, and it could provide us with critical tools to appropriately expand them in vivo in order to be able to modulate in

vivo repair and regeneration processes without the need for in vitro MSC expansion. Some attempts in this direction have been already pursued: for example, the proteasome inhibitor Velcade has been reported to be able to expand the MSC pool in a murine model in vivo [Mukherjee et al., 2008].

A detailed and systematic analysis of the complex network of signaling pathways and cells that regulates the ability of MSCs to self-renew, proliferate, and eventually differentiate in vivo will be undoubtedly important for the identification of pharmacological tools that can be used to regulate the bone marrow MSC population in vivo. Analysis of both mouse models and human diseases has been already proven to be helpful for the identification of crucial signaling pathways in MSC biology [Riminucci et al., 2006; Tripodo et al., 2009]. In our "genomic era," it is not hazardous to assume that global gene expression profiling approaches could be extremely useful to gain insights into the molecular mechanisms that regulate both the size and the differentiation potential of MSCs [Song et al., 2006; Abdallah and Kassem, 2008].

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