MINIREVIEW

Mesenchymal Stem Cells

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Within the bone marrow stroma there exists a subset of nonhematopoietic cells referred to as mesenchymal stem or mesenchymal progenitor cells. These cells can be *ex vivo* expanded and induced, either *in vitro* or *in vivo*, to terminally differentiate into osteoblasts, chondrocytes, adipocytes, tenocytes, myotubes, neural cells, and hematopoietic-supporting stroma. The multipotential of these cells, their easy isolation and culture, as well as their high *ex vivo* expansive potential make these cells an attractive therapeutic tool. In this work we will review the information dealing with the biology of mesenchymal progenitors as it has been revealed mainly by *ex vivo* studies performed with bone marrow-derived cells. The discussed topics include, among others, characteristics of mesenchymal progenitors, evidence for the existence of a vast repertoire of uncommitted and committed progenitors both in the bone marrow and in mesenchymal tissues, a diagram for their proliferative hierarchy, and comments on mobilization, microenvironment, and clinical use of mesenchymal progenitors. Despite the enormous data available at molecular and cellular levels, it is evident that a number of fundamental questions still need to be resolved before mesenchymal progenitors can be used for safe and effective clinical applications in the context of both cell and gene therapies. [Exp Biol Med Vol. 226(6):507–520, 2001]

Key words: mesenchymal stem cells; marrow stromal cells; uncommitted mesenchymal progenitors; human mesenchymal stem cells; characteristics mesenchymal progenitors

Adult Stem Cells

An orderly chain of highly regulated processes involving cell proliferation, migration, differentiation, and maturation leads to the production and sustenance of most cell lineages in adult organisms. The earliest cell type on this chain has been called a stem cell. Together with their extensive capacity for self-renewal, stem cells display a broad potential (often a multipotential) for giving rise to diverse differentiated progenies. In addition to the hematopoietic and intestinal stem cells, considered for many years as paradigms of stem cells, adult organisms contain several other classes of stem cells (Table I).

For a long time, adult stem cells have been considered to be developmentally committed in such a way that they appear restricted to produce specific cell lineages, namely those from the tissue in which the stem cell resides. This rather deterministic concept (i.e., bone marrow forms blood cells, epithelium forms epithelial cells, etc.) has been recently challenged by several bizarre and unexpected findings. Reports have shown that a particular stem cell, besides originating the predicted collection of cells characteristic of the tissue in which they reside in, may also give rise to a set of unacquainted progenitors. Thus, the hematopoietic stem cell, in addition to the production of blood cells, can also originate hepatic oval cells ("blood into liver") (1). In turn, neural stem cells, along with their ability to originate the three main type of cells found in the adult brain (2, 3), also produce early and lineage-committed hematopoietic progenitors ("brain into blood") (4). Mesenchymal stem cells, which originate a variety of mesenchymal phenotypes, can also give rise to nonmesenchymal cells like neural cells ("marrow into brain") (5, 6). Moreover, to suit the elevated demand of precursors that occurs during tissue growth and repair, adult organisms should have the ability to recruit uncommitted progenitors from other tissue sources. This proved to be the case during muscle repair, where mesenchymal stem cells in the bone marrow travel to skeletal muscle (7).

Thus, it seems that in addition to their ability to divide without limits and to give rise to distinctive cells, adult stem cells are remarkably malleable and exhibit a high degree of plasticity. The above is extensive to the rare type of somatic pluripotent stem cell, which has been postulated to be a common precursor of all adult stem cells (8).

The above examples also underline another feature of

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Table I. Human Stem Cells

Stem cell	Location (source)	Cells produced	References
Hematopoietic	Bone marrow	Blood, endothelial, hepatic (oval), and muscle cells	1, 75, 112, 145
Neural	Brain	Neurons, astrocytes, oligodendrocytes, and blood cells	3, 4, 146, 147
Epithelial	Gut, epidermis	All cells in epithelium crypts; all cells in epidermal layers	74, 148–150
Mesenchymal	Bone marrow	Bone, cartilage, tendon, adipose, muscle, marrow stroma, and neural cells	5, 23, 28, 29
Embryonic	Blastocyst inner cell; mass primordial germ cells	All cells	21, 22

stem cells, which is their ability to leave their "tissue" niche and circulate in the blood stream, as occurs with the hematopoetic and mesenchymal stem cells (9–13). However, to express its differentiation program, a circulating stem cell must home into an appropriate microenvironment (14, 15).

The growing body of information regarding what a stem cell can or cannot do (16–18) has directly influenced the abundant attempts to explore their clinical impact (8, 19, 20). It has yet to be established whether their use in cell or gene therapies can match the power envisioned with the utilization of embryonic stem cells (21, 22).

Bone marrow contains not only the hematopoietic stem cell, but also the stem cell for tissues that can roughly be defined as mesenchymatic. The multipotential of mesenchymal stem cells, their easy isolation and culture, as well as their high *ex vivo* expansive potential make these cells an attractive therapeutic tool capable of playing a role in a wide range of clinical applications in the context of both cell and gene therapy strategies. In this work we will review the information dealing with the biology of mesenchymal progenitors after studies performed with cells obtained from bone marrow-derived cultures. It will be evident after reading the review that there are many gaps in our information about several aspects of the biology of mesenchymal progenitors. However, given the enormous promise of these cells to the development of new therapies, there is no doubt that in the near future most fundamental questions will be resolved.

Denominations Utilized to Refer to Mesenchymal Progenitor Cells

In vivo and *in vitro* studies have identified the bone marrow stroma as the source of a multipotent stem cell that gives rise to progenitors for several mesenchymal tissues, including bone, cartilage, tendon, adipose, muscle (23), and hematopoietic-supporting stroma (24, 25). Since their original description, these bone marrow multipotent progenitors were referred to by different names. The original term "colony forming unit-fibroblast (CFU-F)" or "marrow stromal fibroblasts (MSF)" (26–28) has been gradually abandoned and replaced by diverse, still indistinct denominations like "marrow stromal cells (MSC)" (29), "mesenchymal stem cells (MSC)" (23), or mesenchymal progenitor cells (30). Although most denominations reflect a semantic rather than a functional issue, in this review we will use the generic term mesenchymal progenitor cells (MPC), which applies not only to the stem cell *per se*, but to a vast repertoire of committed progenitors exhibiting at least more than one differentiation potential and described to be present both in the bone marrow as well as in several mesenchymal tissues (25, 31–33).

It is necessary to emphasize that the denomination "marrow stromal cells" has also been utilized to design hematopoietic-sustaining monolayers of long-term marrow stroma or Dexter-type cultures (34). However, culture conditions, evolving phenotypes, differentiation potential, and secretion products of the above cells are not analogue, and in fact, are quite dissimilar to that of MPC (24, 35).

Bone Marrow-Derived Mesenchymal Progenitor Cells

In the following sections we will discuss data related to the general characteristics of bone marrow-derived MPC cultures, as well as evidence for the existence of uncommitted and committed mesenchymal progenitors and their proliferative hierarchy.

Characteristics of MPC. Bone marrow stroma is the most recurrent tissue source utilized in growing mesenchymal progenitors. In the case of human MPC, the starting material frequently consists of aliquots of bone marrow obtained from normal donors undergoing marrow aspiration for purposes of allogeneic marrow transplantation. After plating low-density mononuclear cells in a basal medium supplemented with selected batches of fetal bovine serum (29, 30), the evolving population of plastic-adherent cells is considered the primary *ex vivo* source of MPC.

By light or phase contrast microscopy, MPC cultures display a rather homogenous population of fibroblast-like cells (26, 36). Cell cycle studies revealed that while a small fraction of MPC are actively engaged in proliferation (approximately 10% at S + G2 + M), the vast majority of cells are standing at the Go/G1 phase of the cell cycle (30). Although check points and length of each phase of the cell cycle have not been determined, the high percentage of Go/G1 cells suggests a high competence of MPC to differentiate (37). Moreover, the Go/G1 population of MPC in-

cludes a minor and variable subset of resting quiescent cells, as evidenced by RNA and DNA content (30) or by FACS analysis of size and granularity (33).

After subcultivation, MPC exhibit a large but highly variable expansive potential. While some preparations of MPC can be expanded through over 15 cell doublings, others cease replicating after about four cell doublings (38–40). The nature of this conflict may arise from several determinants, among them the procedure used to harvest the marrow (38–41), the low frequency of MPC in marrow harvests (2–5 MPC per 1×10^6 mononuclear cells) (42), and the age or condition of the donor from which MPC were prepared (39, 43). Despite the high *ex vivo* expansive potential, MPC do not loose (after moderate subcultivation) their normal karyotype and telomerase activity (44). However, extensive subcultivation impairs cell function by the onset of evident signs of senescence (39) and/or apoptosis (30).

The development of a series of monoclonal antibodies raised towards surface MPC antigens (45, 46), along with other antibodies developed to characterize bone marrow stromal cells (47–49), has been crucial for the immunophenotyping of these cells. Results have shown that the antigenic phenotype of MPC is not unique, but borrows features of mesenchymal, endothelial, epithelial, and muscle cells (Table II). MPC do not express the typical hematopoietic antigens, CD45, CD34, and CD14 (30, 44).

An extended cytokine expression profile has been described for MPC. As seen in Table II, MPC produce several hematopoietic and nonhematopoietic growth factors, interleukins, and chemokines. While many of these cytokines are constitutively produced, others are only expressed after stimulation (50). In addition, MPC express several cytokines and growth factors receptors (Table II). All together, these data put in evidence that mesenchymal progenitors in the bone marrow contribute to the formation and function of a stromal microenvironment, which produces inductive/regulatory signals not only for MPC but also for the development of hematopoietic progenitors and other nonmesenchymal stromal cells present in the bone marrow (24, 51–53). This proposed dynamic participation of MPC in the marrow microenvironment is strengthened by data showing they produce a vast array of matrix molecules, including fibronectin, laminin, collagen, and proteoglycans (29, 35, 44), and that they express several counter-receptors associated with matrix- and cell-to-cell adhesive interactions (Table II). Of particular relevance is the strong expression of CD44 (30, 44), a receptor for various ligands like hyaluronan and osteopontin, which plays a central role in the organization of the extracellular matrix in the marrow or in the bone, respectively (54, 55).

Several *in vitro* studies have been conducted to assess the differentiation potential of MPC, as well as to set up culture conditions, differentiation stimuli, and methods for the identification of each ultimate differentiated phenotype. A summary of this information is provided in Table III. The above information, supported by *in vivo* studies, demonstrates that bone marrow-derived MPC develop into terminally differentiated phenotypes, like those forming bone (28, 56–59), cartilage (57, 58, 60), tendon (61, 62), muscle (7), neural (5), and adipose tissues (25, 44), or hematopoietic-supporting stroma (28).

Table II. Main Characteristics of Bone Marrow-Derived Mesenchymal Progenitors: Expression of Specific Antigens, Cytokine Receptors, and Adhesion Molecules, and Production of Cytokines and Matrix Molecules

Marker type	Designation	References
Specific antigens	SH2, SH3, SH4 STRO-1 α-smooth muscle actin MAB1740	30, 45, 47, 48
Cytokines and growth factors	Interleukins: 1α, 6, 7, 8, 11, 12, 14, and 15 LIF, SCF, Flt-3 ligand GM-CSF, G-CSF, M-CSF	24, 50
Cytokine and growth factor receptors	IL-1R, IL-3R, IL-4R, IL-6R, IL-7R, LIFR, SCFR, G-CSFR, IFNγR, TNFIR, TNFIIR, TGFβIR, TGFβIIR, bFGFR, PDGFR, EGFR	30, 44, 49, 151
Adhesion molecules	Integrins: αvβ3, αvβ5 Integrin chains: α1, α2, α3, α4, α5, αv, β1, β3, β4 ICAM-1, ICAM-2, VCAM-1, ALCAM-1, LFA-3, L-selectin, endoglin, CD44	30, 44, 152
Extracellular matrix	Collagen type I, III, IV, V, and VI Fibronectin, laminin Hyaluronan, proteoglycans	29, 30, 35

Uncommitted Mesenchymal Progenitors in MPC Cultures. When cultures of bone marrow-derived MPC are examined on the basis of cellular proliferative status, they appear to be nonhomogenous. The work performed by Colter *et al.* (33) has shown that in stationary cultures of bone marrow, MPC subsist a minor population of small and agranular cells (RS-1 cells) with a low capacity to generate colonies and nonreactive to the cell cycle-specific antigen Ki-67. Quiescent RS-1 cells express an antigenic profile that is different from that displayed by the most abundant, fast-growing, and committed precursors (mMSC's) found in expanded cultures of MPC. By studying a precursor-product relationship between RS-1 and mMSC cells, the authors came to the conclusion that the high expansive capacity of mMSCs depends on the presence of RS-1 cells. In turn, RS-1 cells may cycle under stimulation of factors secreted by the most mature mesenchymal progenitor cells. Thus, it seems that RS-1 cells may represent

Table III. Differentiation Potential of Bone Marrow-Derived Mesenchymal Progenitors *in vitro:* Stimuli, Molecular, and Cellular Markers

Differentiation to:	Stimuli	Terminal phenotype identification markers	
		Molecular	Cellular
Adipocytes	Dexamethasone + isobutilmethylxanthine (86) Dexamethasone + isobutilmethylxanthine + indomethacin + insulin (44, 143) Dexamethasone + insulin (32)	PPARγ 2 C/EBPβ aP2 Adipsin Leptin Lipoprotein lipase (44, 153, 154)	Cytoplasmic lipid droplet accumulation (158)
Chondrocytes	TGFβ3 + ascorbic acid (44) TGFβ1 + ascorbic acid (32)	Cbfa-1 Collagen types II and IX Aggrecan (155, 156)	Matrix enriched in proteoglycans and collagen types II and IX (32, 44)
Osteoblasts	Dexamethasone + β-glycerophosphate + ascorbic acid (38)	Cbfa-1 Bone/liver/kidney alkaline phosphatase Bone sialoprotein Osteopontin Osteocalcin Collagen type I (44, 153, 156, 157)	Mineralized matrix formation (36)
Tenocytes	BMP-12 (141)	Collagen type II Proteoglycans (61)	Improved biomechanical properties of implanted tendon (61)
Hematopoietic supporting stroma	Hydrocortisone + horse serum (24) Hemopoietic stem cell (159)	n.d.	Maintain and support hematopoietic differentiation of $CD34^+$ cells (24) Support osteoclastogenesis (159) Support megakaryocytopoiesis and thrombocytopoiesis (53)
Skeletal muscle cells	5-Azacytidine (160, 161)	MyoD Myf 5 and 6 MEF-2 Myogenin MRF4 Myosin (161, 162)	Multinucleated contractile cells (78)
Smooth muscle cells	PDGF-BB (161)	ASMA Metavinculin Calponin h-Caldesmon Later smooth muscle actin (47, 161)	n.d.
Cardiac muscle cells	bFGF (161)	GATA 4 and 6 Cardiac troponin I and C Sarcomeric-actin Slow twitch myosin ANP (161)	n.d.
Astrocytes	DMSO + dexamethasone	Glial fibrillary acidic protein Intermediate filament (5, 6)	Integration into neonatal brain (5)
Oligodendrocytes Neurons	PDGF + EGF + linoleic acid (6)	Galactocerebroside (6) Neurofilament Tubulin BIII Synaptophysin (5, 6)	

an *ex vivo* subset of recycling uncommitted mesenchymal stem cells.

Additional evidence for the presence of uncommitted mesenchymal stem cells in bone marrow-derived cultures of MPC has been provided by the work of Conget *et al.* (unpublished, A. Conget and J.J. Minguell). By following a

different experimental approach, a subset of quiescent cells was isolated by taking advantage of the resistance of growth-arrested cells to the antimetabolite, 5-fluorouracil (5-FU) (63). Cells thus isolated have a low RNA content and a high level of expression of the gene for ODC antizyme, both considered as markers for an unproliferative cellular status (64, 65). Quiescent cells and 5-FU nontreated MPC display a similar antigenic profile except in the distinctive expression by the former cells of CD117, an adult stem cell marker (66, 67). Cells in the quiescent condition seem to represent a population of uncommitted mesenchymal progenitors, since they do not express the osteogenic and adipogenic commitment markers (44) Cbfa-1 and PPAR-γ2, respectively. In turn, after prolonged exposure to fetal bovine serum, the slow-proliferating quiescent cells give rise to committed precursors that grow fast and terminally differentiate.

The existence of progenitors with properties of uncommitted mesenchymal stem cells has also been revealed by the use of clonal cultures of bone marrow-derived MPC (32). Data show that among several clones isolated, one exhibited stem cell properties like a relatively low frequency (1%), a FGF-2 growth dependence (68), and an uncommitted condition evidenced by its inability to differentiate into osteoblasts, chondrocytes, or adipocytes.

All together, the above findings demonstrate that despite *ex vivo* manipulation and subcultivation, cultures of bone marrow-derived MPC contain a rare subset of uncommitted progenitors displaying features of stem cells. Whether these cells represent the *ex vivo* counterpart for the *in vivo* mesenchymal stem cell (26) is not known.

Committed Progenitors in MPC Cultures. In addition to uncommitted mesenchymal progenitors, several classes of committed progenitors are also present in cultures of bone marrow-derived MPC. Nonimmortalized cell clones have been used by Muraglia *et al.* (32) to investigate the nature and properties of committed progenitors present in cultures of bone marrow-derived MPC. When the differentiation potential of the isolated clones was assessed, it was found that while 30% of all clones exhibit a tri-lineage (osteo/chondro/adipo) differentiation potential, the rest exhibit either a bi-lineage (osteo/chondro) or a pure osteogenic potential. Clones with a differentiation potential limited to the osteo/adipo or to the chondro/adipogenic phenotype, as well as pure chondrogenic and adipogenic clones, were not detected. These observations have been extended by other studies using conditionally immortalized clones (25, 69–71). In addition, a clone with properties of a quadripotential mesenchymal progenitor (clone BMC9) has been isolated, which under appropriate conditions differentiates into cells exhibiting phenotypic and functional properties of osteoblasts, chondrocytes, adipocytes, and hematopoietic-supporting stroma (25).

The above discussed data have strengthen the concept that cultures of bone marrow-derived MPC are not homogenous, but consist of an assortment of uncommitted and committed progenitors exhibiting divergent stemness. The latter concept discloses that as progenitors progress towards the terminal phenotype, self-renewal is gradually lost and commitment increases (72, 73).

Proliferative Hierarchy for Mesenchymal Progenitors. *In vivo*, bone marrow has been considered as the site of residency of the uncommitted mesenchymal stem cell, which upon expression of its self-renewal and multidifferentiation potential, commands the continual replenishing of a given supply of mesenchymal cells during the entire lifespan of an organism, both at steady-state and altered conditions (23).

The examples given in the previous sections clearly underline that linearity between the mesenchymal stem cell and its end-stage mature phenotypes does not exist. The concept of proliferative hierarchy has been developed to explain structured cell populations in a tissue involving stem, committed, and mature cells (72, 74, 75). This concept, which is applicable to the vast repertoire of bone marrow-derived mesenchymal progenitors, is based on the assumption that proliferation, differentiation, and maturation are in principle independent; in other words, stem cells divide without maturation, while cells close to functional competence may mature, but do not divide. However, the population of committed cells divide and mature, showing intermediate properties between stem cells and functional mature cells. Therefore, the already discussed notion of stemness of mesenchymal progenitors is not a property of a particular cell type, but a spectrum of capabilities of cell types within a population.

Attempts to draw a scheme for a proliferative hierarchy in mesenchymal progenitors began in 1994 when Caplan, in a very comprehensive paper (23), discussed the experimental and logic basis for the "mesengenic process hypothesis." This has been followed by several models, most of them devoted to proposing a hierarchy for osteoprogenitors, involved in bone cell development (46, 76, 77). Based on the discussed data related with the existence of an uncommitted and various committed progenitors, we propose a diagram for their proliferative hierarchy (Fig. 1). We would like to call attention to the fact that the concept of a mesenchymal stem cell is merely tentative. Since reliable stem cell markers are not yet available, the uncommitted mesenchymal stem cell has been mainly defined in terms of their functional skills, as commented before. Therefore, rephrasing Hall (16), we still do not know whether a "mesenchymal stem cell is a mesenchymal stem cell is a mesenchymal stem cell."

Tissue-Derived Mesenchymal Progenitor Cells

In the next section we will discuss the current evidence for the presence of uncommitted and committed mesenchymal progenitors in cultures started from various mesenchymal tissues.

Muscle-Derived MPC. Work performed with adult human skeletal muscle has demonstrated the existence of

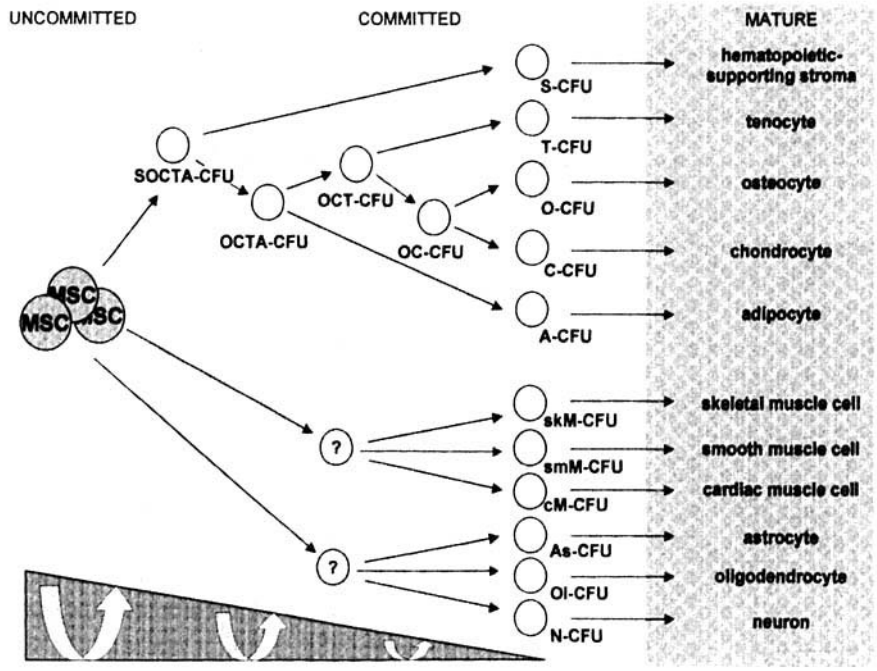

Figure 1. Schematic diagram for the proliferative hierarchy of mesenchymal progenitors. The diagram has been constructed with data from studies performed with expanded human bone marrow-derived mesenchymal progenitor cells. It shows two main compartments containing either uncommitted multipotent mesenchymal stem cells (MSC) or committed mesenchymal progenitors with decreasing stemness, as indicated in the bottom triangle. The committed progenitors are named as colony forming units (CFU) and their differentiation potential into S, hematopoietic-supporting stroma; O, osteoblasts; C, chondrocytes; T, tenocytes; A, adipocytes; skM, skeletal; smM, smooth; cM, cardiac muscle cells; As, astrocytes; Ol, oligodendrocytes; and N, neurons. The third compartment represents, for sake of simplicity, only the mature mesenchymal phenotypes.

cells with properties of early myogenic progenitors (78). In this study it was observed that after tissue enzymatic dissociation and cell cultivation, the resulting primary culture was formed by a mixture of stellate-shaped cells and multinucleated myotubes. After isolation and cultivation of the former cells in medium containing horse serum, cells grow without any sign of differentiation; however, after switching to a medium containing dexamethasone (79), cells started to show signs of differentiation. As judged by morphological and histochemical analysis, the differentiated population included cells with the phenotype of skeletal and smooth muscle, bone, cartilage, and fat. Although the culture conditions used in this work (horse serum and attachment to gelatin) are not those routinely in use for growth and expansion of MPC (30, 39, 44), these results demonstrate the presence in skeletal muscle of committed mesenchymal progenitors. In addition, uncommitted progenitors seem also to be present in muscle. In an attempt to follow the dynamic of myoblast transplantation in the murine system, it was shown that a minority of muscle-resident cells are responsible for new muscle formation (80). The minor cellular subset, which contains slowly dividing cells in culture but rapidly after grafting, probably represents uncommitted mesenchymal stem cells that persist in the environment of the recipient muscle. These results are in agreement with previous reports documenting the existence of such cells (81–83) that seem to be different from muscle satellite cells, classically considered as the muscle stem cell (84). The uncommitted stage of the skeletal muscle-resident mesenchymal progenitor, as well as that of the myogenic cell line C2, are further disclosed by their persistence as undifferentiated mononuclear cells, even after exposure to differentiation stimuli (81).

Not only cells from skeletal muscles, but from other muscles such as the heart, seem to exhibit properties of mesenchymal progenitors. It has been reported that cultures of neonatal rat heart gives rise to a population of adherent stellate cells, which upon incubation with dexamethasone, generate several mesenchymal phenotypes with characteristics of adipocytes, osteoblasts, chondrocytes, smooth muscle cells, skeletal myotubes, and cardiomyocytes (85).

It has not been confirmed whether muscle- and bone marrow-resident mesenchymal stem cells represent the same kind of progenitor. However, the work by Ferrari *et al.* (7) has provided strong evidence that the population of muscle progenitors present in skeletal muscle are derived from uncommitted bone marrow mesenchymal progenitors and are different to muscle satellite cells.

Bone-Derived MPC. Several experimental approaches have been followed to gain insight into the characteristics and differentiation potential of bone-resident mesenchymal progenitors. In one of these studies, four cellular subsets were sorted from primary cultures of normal human bone, according to the differential pattern of expression of the stromal precursor cell marker STRO-1 and the osteoblastic marker alkaline phosphatase (ALP) (76). The STRO-1^+/ALP^- subset exhibited a preosteoblastic phenotype, as evidenced by reduced ability to form a mineralized bone matrix and by the lack of expression of bone sialoprotein, osteopontin, and parathyroid hormone receptor. The other subsets correspond to intermediate and fully differentiated osteoblasts. As expected, after sorting and reculturing, only cells in the STRO-1^+/ALP^- subpopulation were able to give rise to all of the four subsets of STRO-1/ALP cells present in the primary culture. Thus, these results have demonstrated that cultures of human bone are not homogenous, but on the contrary, they include committed osteoprogenitors as well as end-stage differentiated osteoblasts.

Studies with nonimmortalized clonal cell lines derived from human trabecular bone have shown that bipotent- (osteo/adipo) committed progenitors are also present in bone cultures (86). The differentiation pathway taken by these cells is highly modulated by a variety of factors, including long chain fatty acids, drugs, IL-1β, TNF-α, and/or TGF-β (86).

Additional evidence in unveiling the nature of other repositories of progenitors in cultures from bone have been obtained by studies using isolated cell populations. The fetal rat calvaria clone RCJ 3.1 differentiate in a time-dependent sequence into four mesenchymal phenotypes. This progression, which was elicited by ascorbic acid, β-glycerophosphate, and dexamethasone, gave rise to multinucleated muscle cells (Days 9 and 10), adipocytes (Day 12), chondrocytes (after Day 16), and mineralized bone nodules (after Day 21) (79, 87). Together, it has been shown that a population of cells from fetal rat periosteum isolated on the basis of granularity (S cells) exhibit various properties of an uncommitted mesenchymal progenitors. Thus, S cells are slow cycling, do not express differentiation-associated markers, and when grown in culture, generate cartilage, adipose, smooth muscle, and bone phenotypes (31, 88).

Thus, studies utilizing distinct experimental approaches have established that cultures of bone-derived MPC contain uncommitted mesenchymal progenitors as well as committed osteoprogenitors. All together, this evidence puts forward the contention that uncommitted mesenchymal stem cells are not only located in the marrow, but are also ubiquitously positioned in bones where, under appropriate stimuli (microenvironment?), may self-renew, commit, and generate cells exhibiting the phenotypic and functional characteristics of the resident tissue (89).

Cartilage-Derived MPC. *In vivo*, articular cartilage has a limited capacity for repair (90). It has been suggested that despite the presence of cells capable of developing into a correct chondrocytic phenotype, their number or the amount of regulatory factors is limited in the repair tissue (91–95). However, it is not clear whether the "repair cell" corresponds to a chondrocyte-committed progenitor located in the cartilage or to an osteo/chondrocyte-committed progenitor recruited from a noncartilagenous tissue (79, 96–98).

Tendon-Derived MPC. Few studies have addressed the issue of the presence of tendon-resident mesenchymal progenitors. By developing a method for the serial culture of tenocytes from juvenile rabbit Achilles tendon, it was shown that cells in primary and first passage cultures retained the expression of tenocyte differentiation markers like collagen type I and decorin. However, after successive passaging, despite the fact that cells are healthy and with no evidence of senescence, tenocytes started to display a modulated phenotype (99). Regardless of the abundant information on factors that modulate the growth of tendon cells *in vitro* (100–104), there is no data on the tissue origin or the commitment condition of tendon-resident precursors.

Adipose Tissue-Derived MPC. Adipose tissue stromal cells contain adipocyte progenitors at various stages of maturity, including the stromal-vascular (SV) cells, considered as the less differentiated tissue-resident adipocyte progenitor. *In vivo* SV cells are induced to proliferate and differentiate into mature adipocytes during cold acclimation (105) and after caloric excess (106). Both SV cells and bone marrow-derived MPC can be induced to differentiate into adipocytes by glucocorticoids, IGF-I, and insulin (106). Therefore, SV cells are a class of fat-resident-committed mesenchymal progenitors, exhibiting at the least a bipotent differentiation potential, since they can differentiate into adipocytes or chondrocytes (107). Additional evidence for the extensive differentiation potential exhibited by fat-resident progenitors came from studies using cloned cells isolated from fat bone marrow, which demonstrated their capability to differentiate into adipocytes or osteoblasts (108).

Vascular-Derived MPC. Most, if not all, vessels develop from an endothelial tube that subsequently acquires a coating formed by vascular smooth muscle cells/pericytes (vSMC), which in turn develop from a undifferentiated perivascular mesenchymal progenitor (109). Perivascular mesenchymal progenitors exhibit many features of bone marrow-derived MPC, like expression of α-smooth muscle actin (ASMA), PDGF-mediated growth stimulation via a PDGF receptor, and a differentiation potential following a typical smooth muscle pathway (23, 47, 109). Thus, vSMC represent a vascular-resident mesenchymal progenitor with the potential to differentiate, at least, into the smooth muscle lineage.

Mesenchymal Progenitor Development: Mobilization and Microenvironment(s)

The concept that a bone marrow-resident-uncommitted mesenchymal stem cell gives rise to all mesenchymal lineages in distant tissues (23, 26, 29, 36) is supported by evidence coming from analysis undertaken both *in vivo* and *in vitro*. However, data quoted in the previous sections show that committed mesenchymal precursors with different stemness or even uncommitted mesenchymal stem cells are located in marrow-distant mesenchymal tissues, as seems to be the case in muscle and bone. These facts renew the question as to whether a tissue-resident progenitor has always existed in that particular tissue or has been recruited as such or as a less committed precursor from the bone marrow or another mesenchymal tissue. Although the limits for the above circumstances are difficult to set, current information suggests that progenitor recruitment occurs during the growing period of an organism (110, 111), as well as in adult life during tissue repair (7, 89, 98). Accordingly, two main issues are raised. The first one is related to the route(s) taken by the precursor cell in case they originate from another tissue or another area in the same tissue, and the second one relates to the homing and fate within a particular

tissue of mesenchymal progenitors. These two aspects will be discussed in the following sections.

Mobilization of Mesenchymal Progenitors. Bone marrow stroma, the site of residence of the uncommitted multipotent mesenchymal stem cell, feeds progenitors into distant mesenchymal tissues (23). Therefore, one may assume that for destination into other tissues, the mesenchymal stem cell must leave the marrow stroma as such or after undergoing either self-renewal and commitment. The latter may take place by a successive traffic throughout distinct stromal niches that regulate stem cell development (15, 18, 112). As a corollary, cell-to-cell or cell-to-matrix interactions between mesenchymal progenitors and stromal components should loosen up, thus facilitating the egress of the progenitor into the blood stream. Therefore, peripheral blood should represent a transit compartment for mesenchymal progenitors in the search of their final destination: a proper microenvironment in a distant tissue where they can home, expand, and further differentiate.

Whether adult mesenchymal progenitors circulate in peripheral blood is an open issue. In the murine model, CFU-Fs circulate in the blood and represent a stromal cell population that can migrate into various tissues (113). In humans, mesenchymal progenitors have been detected in peripheral blood from breast cancer patients after growth factor mobilization of hematopoietic stem cells (11). Moreover, by using a positive selection procedure, a population of adherent cells that originate colonies of mesenchymal progenitors has been isolated from peripheral blood (13). However, circulating mesenchymal progenitors in human blood have not been detected in other studies (114, 115). Besides the dissimilar experimental conditions that may have affected the interpretation of the above results, it seems important to determine whether mesenchymal progenitors can be found in the blood of healthy or unhealthy individuals, as well as after marrow stimulation (7, 116). The latter case occurs with the hematopoietic stem cell, which is released from the bone marrow into the blood stream only after stimulation by drugs or growth factors (117).

Recent data show that in addition to adult bone marrow and other mesenchymal tissues, umbilical cord blood is also a source of mesenchymal progenitors (12). These "in motion" mesenchymal cells display many common features with adult human bone marrow-derived mesenchymal progenitors, like adhesion to plastic, morphology, expression of cell membrane and cytoplasmic antigens, and a potential to differentiate into osteo/chondrogenic and adipogenic phenotypes. Moreover, the identification in cultures of mesenchymal cells from cord blood of a subset (5%–10%) of quiescent cells suggests that an uncommitted mesenchymal progenitor circulates during gestation. The inverse correlation between content of mesenchymal progenitors in cord blood and gestational age, a trend also observed for hematopoietic progenitors (10), suggests that mesenchymal cells travel from fetal sites into other tissues early during development (12).

The preceding as well as other evidence (7, 27, 57) give strength to the existence of a sort of "long-distance" traffic of mesenchymal progenitors via the blood stream. However, "short distance" or "local" traffic of mesenchymal progenitors has been described to occur within a tissue during cartilage repair (97, 98), muscle regeneration (80), migration throughout forebrain and cerebellum (5), and gingiva and periodontal cell differentiation into osteoblasts (118).

Microenvironment(s) for Mesenchymal Progenitors. Maintenance of stem cell compartment ultimately depends on cell autonomous regulators modulated by external signals. Such intrinsic regulators include, among others, factors controlling asymetric cell division, expression of genes related with the uncommitted and committed stages, and clocks that set the number of rounds of cell division. In turn, extrinsic signals that control stem cell fate collectively make up the stem cell microenvironment or niche (119). This niche involves a complex interplay of short- and long-range signals between uncommitted and committed progenitors and between them and neighboring cells.

The nature and properties of an adult microenvironment for the hematopietic, neural, and ephithelial stem cells have been accomplished by several studies (15, 112, 120–122). However, there is no comprehensive data on the characteristics and properties of a microenvironment for mesenchymal progenitors, both in the bone marrow and in mesenchymal tissues. Rather, there is increasing information on a variety of selected modulators that seem to control mesenchymal progenitor development (Table IV). One may assume that any of these molecules, along with diverse cell types (mesenchymal and nonmesenchymal) and their products (growth factors and matrix molecules), can establish vicinity and temporal relationships that make up the framework for mesenchymal microenvironment(s). The vast repertoire of mesenchymal progenitors identified both in the bone marrow and in mesenchymal tissues underscore the need to gain more information not only in the description of a bone marrow microenvironment, but in "local" or mesenchymal tissue microenvironments. The molecular and cellular analysis of such microenvironments will help to understand the fate of each mesenchymal progenitor within a particular tissue, as it has been suggested by several *in vivo* studies (5, 68, 81, 123–126). Together, it may be meaningful to distinguish between a physiologically ongoing versus an injury-derived microenvironment. This distinction should be important in terms of specification of cell phenotype (121) in normal and injury-derived microenvironments. It is without doubt that improvement in the knowledge of mesenchymal microenvironments should have a profound impact in the clinical utilization of mesenchymal progenitors.

Clinical Trials using Mesenchymal Progenitors

Given the promising features of adult stem cells for the development of new cell therapies (8, 77), researchers in the

Table IV. Modulators of Bone Marrow-Derived Mesenchymal Progenitors Differentiation

Modulator	Promotes differentiation to:	Precludes differentiation to:	References
1,25-Dihidroxyvit D3	Osteoblasts	Adipocytes	69, 163
Prostaglandin E2	Osteoblasts		164
GH	Osteoblasts		165
LIF	Adipocytes		166
IL-6	Osteoblasts		167
Leptin	Osteoblasts	Adipocytes	168
	Osteoblasts		
TGFβ1	Chondrocytes	Myotubes	32, 169
TGFβ3	Chondrocytes		44
	Osteoblasts		
BMP2	Adipocytes	Myotubes	141, 153, 169, 170
BMP4	Osteoblasts		171
	Tenocytes		
BMP12	Chondrocytes		141
Minimally oxidized low-density lipoprotein	Adipocytes	Osteoblasts	172

field of mesenchymal progenitors have pursued a broad range of investigations to give impulse to their therapeutic utilization. However, a main issue to be resolved is whether mesenchymal progenitors are transplantable, and in addition, what type of progenitor (uncommitted versus committed) is transplantable. These questions will help to decide whether direct loading (injection or implant) or systemic infusion is the best route for mesenchymal progenitor delivery. The former case probably is best suited with a clinical strategy oriented to augment local repair or regeneration of bone (56, 58, 59), cartilage (127), or tendon (61). On the other side, blood delivery of mesenchymal progenitors may be useful in recovering not only a local, but also a systemic dysfunction of a tissue by re-starting their own developmental program. Particularly, in the case of bone marrow one may speculate that the infusion of mesenchymal progenitors followed by a selective homing into marrow stromal sites (51, 128) can result in the increase or improvement of the function of hematopoietic-supporting stroma, which in turn may facilitate engrafment and differentiation of hematopoietic stem cells (77, 129, 130).

The issue of transplantabilty has been addressed in several studies; however, results are rather contradictory in establishing the origin (host or donor) of mesenchymal progenitors after allogeneic transplantation of marrow harvests (42, 43, 131–134). Contradiction probably arises from the use of different experimental conditions, among them procedures to harvest the marrow (40, 41), methods to type and measure progenitor content in marrow harvests (42), and patient's marrow status to which cells are transplanted (39, 43). In addition, it appears essential to take into account whether the material to be transplanted has to be *ex vivo* expanded to increase the number of progenitors. This may be an important issue, since progenitor stemness and function diverge as cells are subcultivated (30, 39). In the hematopoietic and muscle systems it has been demonstrated that stemness of the grafted cells determines either the long- or short-term repopulation of the damaged tissue (80, 135). It has yet to be established whether the same occurs after transplantation of *ex vivo* expanded mesenchymal progenitors. Are all mesenchymal progenitors (uncommitted and/or committed) competent to sustain both a long- and short-term mesengenesis?

The first clinical trials reported have revealed that the systemic infusion of *ex vivo* expanded autologous mesenchymal progenitors is feasible and safe in the short-term (129, 136). On the other hand, it has been demonstrated that allogeneic bone marrow transplantation (considered as a common source of hematopoietic and mesenchymal progenitors) in children with osteogenesis imperfecta results in impressive histological changes in trabecular bone, which are indicative of new dense bone formation. In addition, increased growth rate and reduced frequencies of bone fracture were also observed (19). These changes, detected 3 months after marrow transplantation, were associated with the engraftment of functional mesenchymal progenitors from the transplanted marrow (137).

Conclusions and Future Directions

The last 5 years have been the scene of a substantial improvement in our understanding of the biology and the potential clinical utilization of adult mesenchymal progenitors. Despite the abundant data on their isolation, culturing, expansion, and differentiation potential, there is still few comprehensive data on mesenchymal progenitor stemness, both *in vivo* and after *ex vivo* cultivation. While several molecular markers are available for committed progenitors and the end-stage phenotypes, at present there are no reliable cell markers to identify the mesenchymal stem cell, *per se*. The few attempts performed to isolate and characterize the mesenchymal stem cell are based on methods that make use of their functional capabilities, which in turn can only be assessed by testing them, which itself may alter the stem cells. There is no doubt that a better characterization of the

uncommitted mesenchymal stem cell in the marrow as well as in distant tissues is an immediate aim in the biology of mesengenesis. Moreover, the terminology used to describe the repertoire of uncommitted and committed progenitors is still not well defined and has been used unrestrained, which leads to confusion.

However, this lack of information has not been an obstacle in pursuing the therapeutic utilization of these cells, which represents an attractive option for a wide range of clinical applications in the context of both cell and gene therapy strategies. As an integral component of the marrow stroma, mesenchymal progenitor transplantation alone or in conjunction with hematopoietic progenitors would facilitate the engraftment of the hematopoietic stem cell after myeloablative therapy. Also, it has to be determined whether mesenchymal progenitors have the potential to replace chemotherapy- or disease-associated damaged stroma (43), or perhaps their utilization may be beneficial in the management of other diseases (138, 139).

Simultaneously, as precursors of several mesenchymal lineages, mesenchymal progenitors are envisioned as a proper therapy to attenuate or correct disorders of several mesenchymal tissues, among them osteogenesis imperfecta, osteoporosis, osteoarthrosis, meniscectomy, and muscular dystrophy. In this respect, recent studies showing the feasibility of adeno- or retroviral-mediated gene transfer of reporter or therapeutic genes into mesenchymal progenitors will greatly contribute to the clinical utilization of these cells (140–143). For the near future we anticipate a rapid closure of many gaps in our knowledge of the biology of mesenchymal progenitor cells, which may facilitate the development of phase II and III clinical trials for new therapeutic alternatives (144). Thus, as it has been recently insinuated, mesenchymal progenitors are "no longer second class marrow citizens" as compared with hematopoietic progenitors, the paradigm of bone marrow cells (137).

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