Review

Mesenchymal stromal cells

Biology of adult mesenchymal stem cells: regulation of niche, self-renewal and differentiation

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Published: 19 February 2007
This article is online at http://arthritis-research.com/content/9/1/204

Arthritis Research & Therapy 2007, **9:**204 (doi:10.1186/ar2116)

Abstract

Recent advances in understanding the cellular and molecular signaling pathways and global transcriptional regulators of adult mesenchymal stem cells have provided new insights into their biology and potential clinical applications, particularly for tissue repair and regeneration. This review focuses on these advances, specifically in the context of self-renewal and regulation of lineage-specific differentiation of mesenchymal stem cells. In addition we review recent research on the concept of stem cell niche, and its relevance to adult mesenchymal stem cells.

Introduction

Since the seminal identification of mesenchymal stem cells (MSCs) as colony-forming unit-fibroblasts (CFU-Fs) by Friedenstein and colleagues in 1970 [1] and the first detailed description of the tri-lineage potential of MSCs by Pittenger and colleagues [2], our understanding of these unique cells has taken great strides forward. MSCs have great appeal for tissue engineering and therapeutic applications because of their general multipotentiality and relative ease of isolation from numerous tissues. This review highlights recent discoveries in the areas of MSC self-renewal, differentiation, and niche biology, and presents molecular signaling and mechanistic models of MSC development.

MSC markers

Plastic-adherent multipotent cells, capable of differentiating into bone, cartilage and fat cells (among others), can be isolated from many adult tissue types. However, even if isolated by density-gradient fractionation, they remain a heterogeneous mixture of cells with varying proliferation and differentiation potentials. Although acceptable for cell-based therapeutic applications, a rigorous understanding of the MSC requires a better definition of what an MSC is. Many attempts have been made to develop a cell-surface antigen profile for the better purification and identification of MSCs. Particularly important is whether MSCs isolated from different tissues are identifiable by the same immunophenotype. Table 1 provides information on 16 surface proteins reported in various studies. Most of the studies focused on MSCs from human and mouse bone marrow, but some examined MSCs from other organs. There is a surprisingly small amount of variation between populations, even among cells isolated from different sources. It is also noteworthy that the mouse bone marrow-derived multipotent adult progenitor cell (MAPC) subpopulation [3], reported to have more differentiation potential than the MSC population as a whole, does not express specific, known surface markers.

Negative markers

There is a consensus that MSCs do not express CD11b (an immune cell marker), glycophorin-A (an erythroid lineage marker), or CD45 (a marker of all hematopoietic cells). CD34, the primitive hematopoietic stem cell (HSC) marker, is rarely expressed in human MSCs, although it is positive in mice. CD31 (expressed on endothelial and hematopoietic cells) and CD117 (a hematopoietic stem/progenitor cell marker) are almost always absent from human and mouse MSCs. Currently, the thorn in the side of the MSC biologist is the lack of a definitive positive marker for MSCs; there is a myriad

αSMA = α-smooth muscle actin; bHLH = basic helix–loop–helix; BMP = bone morphogenetic protein; CFU-F = colony-forming unit-fibroblast; ECM = extracellular matrix; FGF = fibroblast growth factor; GDF = growth and differentiation factor; HAT = histone acetyltransferase; HGF = hepatocyte growth factor; HSC = hematopoietic stem cell; LIF = leukemia inhibitory factor; MAPK = mitogen-activated protein kinase; MSC = mesenchymal stem cell; MSK = mitogen- and stress-activated protein kinase; PCAF = p300/CBP-associated factor; PDGF = platelet-derived growth factor; PPAR = peroxisome proliferator-activated receptor; TAZ = transcriptional coactivator with PDZ-binding motif; TGF-β = transforming growth factor-β; TIP = tension-induced/-inhibited protein; TNF-α = tumor necrosis factor-α; Wnt = mammalian homologue of *Drosophila* wingless.

Table 1

Surface antigens commonly identified during isolation of mesenchymal stem cells (MSCs)

		Number of populations reported with specified antigen levels[b]						
		Human MSCs[c]			Murine MSCs[c]			
Marker type	Surface antigen[a]	+	+/–	–	+	+/–	–	References
Positive	Stro-1	7	1	2	0	0	0	4-7,66,82-84
	CD13	5	0	0	1	0	1	2,12,84-87,89-90
	CD29	5	0	0	11	0	0	2,12,63,84-87,90
	CD44	11	0	1	10	1	0	2,63,82,84-87,90-91
	CD73	5	0	0	0	0	0	2,10,83-85
	CD105	7	0	0	1	0	0	2,10,12,83-87
	CD106	4	0	2	4	1	0	2,5,83-84,86-89
Negative	CD11b	0	0	3	0	1	5	2,82,86-88,90
	CD31	0	3	10	0	0	6	2,82,84-91
	CD34	1	1	10	5	6	3	2,12,63,82,84-89,91
	CD45	0	0	11	0	0	6	2,82,84-91
	CD117	0	2	3	1	1	13	2,63,82,87-90
Variable	Sca-1	0	0	0	6	5	4	63,87-88,90
	CD10	6	0	5	0	1	0	82,85-87,89
	CD90	11	1	1	2	4	10	2,12,63,82,84-85,87-91
	Flk-1	2	1	1	0	0	5	82,88-89

[a]Antigen chosen if tested in at least 4 MSC populations from the 19 papers reviewed; [b]number of MSC populations (isolated from various tissues from human or mouse) reported in these studies to be mostly positive (+), somewhat positive (+/–), or negative (–); [c]MSCs isolated primarily from bone marrow but also from fat, skin, thymus, kidney, muscle, liver, lung, and placenta.

of reported positive markers, with each research group using a different subset of markers. Without a definitive marker, *in vivo* studies on cell lineage and niche are difficult. Only the most characterized and promising markers with the highest specificities are described below.

Positive markers

Stro-1 is by far the best-known MSC marker. The cell population negative for Stro-1 is not capable of forming colonies (that is, it does not contain CFU-Fs) [4]. Negative selection against glycophorin-A, together with selection of strongly Stro-1-positive cells, enriches CFU-Fs in harvested bone marrow cells to a frequency of 1 in 10 [5]. Stro-1-positive cells can become HSC-supporting fibroblasts, smooth muscle cells, adipocytes, osteoblasts, and chondrocytes [6], which is consistent with the functional role of MSCs. In addition, expression of Stro-1 distinguishes between two cultured populations of MSCs that have different homing and HSC-supportive capacities [7]. However, Stro-1 is unlikely to be a general MSC marker, for three reasons: first, there is no known mouse counterpart of Stro-1; second, Stro-1 expression is not exclusive to MSCs; and third, its expression in MSCs is gradually lost during culture expansion [5], limiting the use of Stro-1 labeling to the isolation of MSCs and/or their identification during early passages. Because the exact function of the Stro-1 antigen is unknown, it is unclear whether loss of Stro-1 expression alone has functional consequences for MSC stemness. Application of Stro-1 as an MSC marker is therefore best done in conjunction with other markers (see below).

CD106, or VCAM-1 (vascular cell adhesion molecule-1), is expressed on blood vessel endothelial and adjacent cells, consistent with a perivascular location of MSCs (see the 'MSC niche' section below). It is likely to be functional in MSCs because it is involved in cell adhesion, chemotaxis, and signal transduction, and has been implicated in rheumatoid arthritis [8]. CD106 singles out 1.4% of Stro-1-positive cells, increasing the CFU-F frequency to 1 in 3, which are all high Stro-1-expressing cells and are the only Stro-1-positive cells that form colonies and show stem cell characteristics such as multipotentiality, expression of telomerase, and high proliferation *in vitro* [5]. Taken together, these data suggest that Stro-1 and CD106 combine to make a good human MSC marker.

CD73, or lymphocyte-vascular adhesion protein 2, is a 5′-nucleotidase [9]. Although also expressed on many other cell types, two monoclonal antibodies (SH-3 and SH-4) against CD73 were developed with specificity for mesenchymal tissue-derived cells [10]. These antibodies do not react with HSCs, osteoblasts, or osteocytes, all of which could potentially contaminate plastic-adherent MSC cultures. The persistence of CD73 expression throughout culture also supports its utility as an MSC marker.

Other markers

Many other surface antigens are often expressed on MSCs, but they are not highlighted above because of their lack of consistent expression or specificity or because of insufficient data. These include: CD271/NGFR [11], CD105, CD90/Thy-1, CD44, CD29, CD13, Flk-1/CD309, Sca-1, and CD10. (See Table 1 for further details.)

We recommend Stro-1, CD73, and CD106 as the most useful markers, although their functions remain to be determined. Cell migration, cytoskeletal response, and signaling pathway stimulation assays currently used to analyze other MSC membrane proteins may prove to be helpful in studying these markers [12].

MSC self-renewal and maintenance

Self-renewal refers to the biological pathways and mechanisms that preserve the undifferentiated stem state. Genomic arrays have been used to identify putative molecular signatures that maintain the stem cell state, including that of MSCs [13]. Candidate gene approaches have also been successful in understanding how MSCs self-renew (Figure 1).

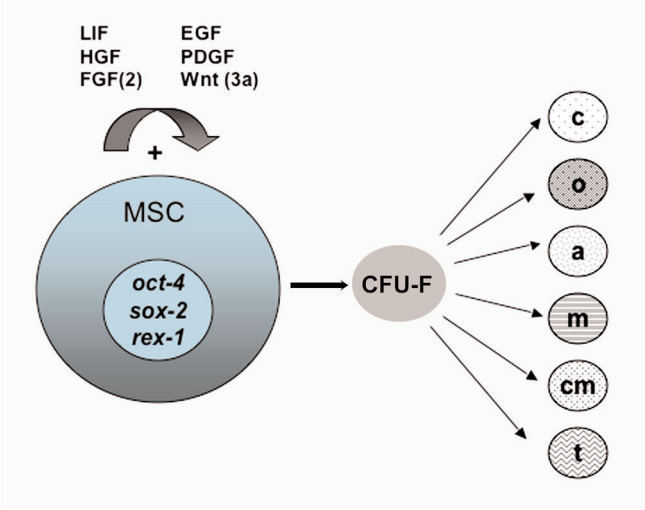
Leukemia inhibitory factor (LIF) [14,15], fibroblast growth factors (FGFs) [16,17], and mammalian homologues of *Drosophila* wingless (Wnts) [18,19], among other growth factors and cytokines, have been implicated in MSC 'stemness' maintenance. These factors have drawn particular focus because of their demonstrated role in the self-renewal of other stem cell types, in the maintenance of undifferentiated embryonic mesenchymal tissue, and/or in dedifferentiation programs, including tumorigenesis.

LIF, a pleiotropic cytokine, maintains the stem state of MSCs [14] and other stem cells [15]. LIF also activates and represses osteoblast and osteoclast activities [20]. The bipotency of LIF suggests that the cellular environment and the developmental stage of the target cell influence its differential responses to LIF. Mechanisms of LIF action in MSC self-renewal are unknown but may involve paracrine crosstalk with neighboring cells [21].

FGF2 maintains the stem state of MSCs from a variety of species by prolonging their viability in culture [16], sometimes in a cell-autonomous fashion [17]. This is reminiscent of the maintenance of undifferentiated limb bud by an FGF4, FGF8, and FGF10 feedforward loop between the apical ectodermal ridge and underlying mesenchyme [22]. Extensive genetic mapping has established causal links between FGF/FGF-receptor allelic mutations and a spectrum of human craniosynostoses and achondrodysplastic syndromes [23], recapitulated in animal models [22]. Target genes of FGF involved in maintaining MSC stemness are not known. It is plausible that an autocrine regulatory loop may underlie FGF self-renewal function, as during vertebrate limb development [23].

Evidence from our laboratory suggests that Wnts may also regulate MSC maintenance [19], as they do in the self-renewal of hematopoietic, neural, intestinal, and skin stem cells [18]. Wnt3a treatment increases adult MSC proliferation while inhibiting their osteogenic differentiation [19]. However, discerning the exact involvement of Wnts is complicated by their pleiotropic effects. Examples of canonical Wnt functions include the promotion of long-term culture expansion of stem cells, increased *in vivo* reconstitution of hematopoietic lineages, and Wnt3a-specific maintenance of skin and intestinal stem cell populations [18]. Because stem cells may share signaling mechanisms with cancer cells that arise from deregulated differentiation programs, the sustained β-catenin expression observed in some colon carcinomas [24] suggests a downstream involvement of β-catenin in Wnt regulation of MSC self-renewal.

Figure 1



Mesenchymal stem cell self-renewal and cytodifferentiation. Extracellular signaling factors, including growth factors and cytokines, demonstrated to promote and/or maintain mesenchymal stem cell (MSC) self-renewal, *in vitro*. Gene markers characteristic of MSC self-renewal include *oct-4*, *sox-2*, and *rex-1*. LIF, leukemia inhibitory factor; EGF, epidermal growth factor; HGF, hepatocyte growth factor; PDGF, platelet-derived growth factor; FGF, fibroblast growth factor; CFU-F, colony forming unit-fibroblast; c, chondroblast; o, osteoblast; a, adipoblast; m, myoblast; cm, cardio-myoblast; t, tenoblast.

MSCs from a variety of mammalian species also express the embryonic stem cell gene markers *oct-4*, *sox-2*, and *rex-1*, among others [25]. Recent chromatin immunoprecipitation on chromatin immunoprecipitation array studies suggest that some Polycomb chromatin-associated proteins are involved globally in maintaining the repression of differentiation genes [26]. Thus, Polycomb proteins may indirectly maintain *oct-4*, *sox-2*, and *rex-1* activation in MSCs; alternatively, Trithorax proteins, which complement Polycomb proteins [27] by maintaining the activation of homeotic genes, may directly regulate the expression of *oct-4*, *sox-2*, *and rex-1*. Biochemical studies linking stemness gene expression with chromatin-associated proteins will be an interesting future avenue of research.

Several other exciting areas of MSC biology that are beyond the scope of this review have recently begun to be explored. These areas concern the regulation of other cell types by MSCs, including MSCs as trophic mediators [28] and the immunomodulatory effects of MSCs [29].

MSC differentiation

The identification of specific signaling networks and 'master' regulatory genes that govern unique MSC differentiation lineages remains a challenge. The ability to modulate biological effectors to maintain a desired differentiation program, or possibly to prevent spurious differentiation of MSCs, is needed for effective clinical application, as in tissue engineering and regeneration. Some of the recently discovered lineage-restrictive molecular regulators and their mechanisms of action will be reviewed here.

Chondrogenesis

Chondrogenic differentiation of MSCs *in vitro* mimics that of cartilage development *in vivo.* Expression markers associated with chondrogenesis have been positively characterized in MSC-derived chondrocytes, including transcription factors (sox-9, scleraxis) and extracellular matrix (ECM) genes (collagen types II and IX, aggrecan, biglycan, decorin, and cartilage oligomeric matrix protein) [30,31]. However, the specific signaling pathways that induce the expression of these benchmark chondrogenic genes remain generally unknown. Naturally occurring human mutations and molecular genetic studies have identified several instructive signaling molecules, including various transforming growth factor-β (TGF-β) [32], bone morphogenetic protein (BMP), growth and differentiation factor (GDF) [33] and Wnt [34] ligands. Recombinant proteins and/or adenoviral infection of MSCs with TGF-β1 and TGF-β3, BMP-2, BMP-4, BMP-6 [35], BMP-12 [36], BMP-13 [37], and GDF-5 have been shown to rapidly induce chondrogenesis of MSCs from a variety of mesodermal tissue sources (reviewed in [31]). Upon receptor binding, TGF-βs and BMPs signal through specific intracellular Smad proteins and major mitogen-activated protein kinase (MAPK) cascades, providing levels of specificity that are actively being investigated in MSC differentiation contexts [32,38]. Recent studies into mechanisms of crosstalk between downstream MAPK signaling and Smad effectors have revealed that MAPK substrates include chromatin histone acetyltransferases (HATs) [39]. HATs in turn are directly recruited by Smads and enhance Smad transactivation capability [40]. For example, the p38 MAPK substrate MSK phosphorylates p300-PCAF HATs [39], thereby enhancing their direct binding to and formation of a Smad2/4–HAT complex. This may be a general model of how the two major signaling mediators of the TGF-β and BMP ligands converge synergistically to transactivate target genes of chondrogenesis, with a specificity probably dependent, in part, on the unique combinatorial crosstalk between R-Smads and MAPK pathways.

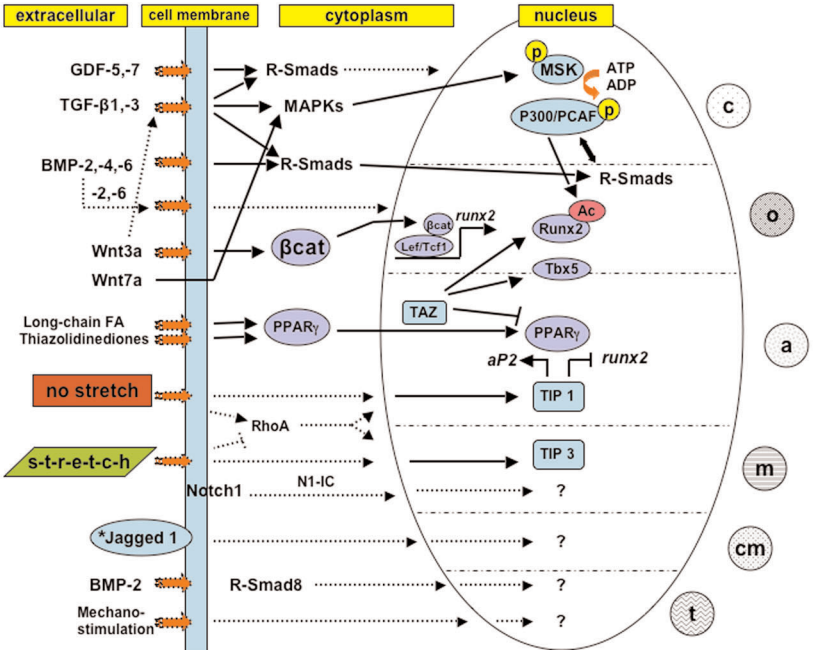
Wnts have an important bipotent modulatory function in chondrogenesis. In murine C3H10T1/2 cells, canonical Wnt3a enhances BMP-2-induced chondrogenesis [41,42]. Wnt3a in turn regulates *bmp2* expression [43], suggesting a feedforward regulatory loop during chondrogenesis. In human MSCs, transient upregulation of Wnt7a also enhances chondrogenesis through various TGF-β1–MAPK signaling pathways, but sustained Wnt7a expression is chondroinhibitory [44]. A recent study in ATDC5 cells revealed that Wnt1 inhibits chondrogenesis through the upregulation of the important mesodermal basic helix–loop–helix (bHLH) transcription factor, Twist 1 [45], perhaps involving negative sequestration of chondrostimulatory factors or direct repression of target genes. Further investigations should focus on the crosstalk between pathways, such as those of TGF-βs and Wnts.

Osteogenesis

BMPs, in particular BMP-2 and BMP-6, strongly promote osteogenesis in MSCs [33,46]. BMP-2 induces the p300-mediated acetylation of Runx2, a master osteogenic gene, which results in enhanced Runx2 transactivating capability. The acetylation is specific to histone deacetylases 4 and 5, which, by deacetylating Runx2, promote its subsequent degradation by Smurf1 and Smurf2, and E3 ubiquitin ligases [47]. Interestingly, the cytokine TNF-α, which is associated with inflammation-mediated bone degradation, also downregulates Runx2 protein levels through increased degradation mediated by Smurf1 and Smurf2. Transgenic TNF-α mice also showed increased levels of Smurf1 and Smurf2, concurrent with decreased Runx2 protein levels [48]. These findings suggest that therapeutic approaches to MSC-based bone tissue engineering, centered on BMPs, Runx2, and histone deacetyltransferases, may enhance existing TNF-α-based immunotherapy of bone diseases.

Wnts have an important modulatory function in osteogenesis. Knockout and dosage compensation in Wnt-pathway-related transgenic animals provide the strongest proof that high levels of endogenous Wnts promote osteogenesis, whereas low levels inhibit osteogenesis [49]. In C3H10T1/2 and

Figure 2



Molecular regulation of mesenchymal stem cell cytodifferentiation programs. Extracellular molecular signaling and mechanical inducers of differentiation transduce effects through putative receptors, channels, and/or other cell-surface-associated mechanisms. Downstream crosstalk of signaling pathways, including that between distinct mitogen-activated protein kinases (MAPKs) and R-Smads, provides a level of specificity that gives rise to unique lineages, such as chondrocytes and osteoblasts. Specificity of lineage differentiation can also result from the recruitment of master transcriptional switches with binary regulation of cell fate, such as TAZ (transcriptional coactivator with PDZ-binding motif). Depending on potentially unique multiprotein complexes that it may form in response to specific upstream signaling, TAZ promotes osteogenesis and inhibits adipogenesis. Furthermore, coregulator subtypes can be invoked, such as tension-induced/-inhibited proteins (TIPs), which regulate adipogenesis and myogenesis. Specific molecular induction/regulation of cardiomyogenic and tenogenic-specific development are as yet largely unknown, with the exception of those depicted. Broken lines, unknown or putative; solid lines, as in published data; *, juxtaposing cell; GDF, growth and differentiation factor; TGF, transforming growth factor; BMP, bone morphogenetic protein; FA, fatty acid; βcat, β-catenin; PPAR, peroxisome proliferator-activated receptor; MSK, mitogen- and stress-activated protein kinase; PCAF, p300/CBP-associated factor; Ac, acetyl; c, chondroblast; o, osteoblast; a, adipoblast; m, myoblast; cm, cardiomyoblast; t, tenoblast.

murine osteoprogenitor cells, canonical Wnt signaling up-regulates *runx2*. Chromatin immunoprecipitation and promoter mutational analyses showed that β-catenin/LEF (lymphoid enhancer binding factor)/TCF1 (T-cell factor 1) occupy a cognate binding site in the proximal *runx2* promoter and may therefore directly regulate *runx2* expression [50]. However, in human MSCs, canonical Wnts decrease osteogenesis [19]. Independently, these observations suggest a mechanistic model of MSC osteogenesis involving crosstalk between BMPs and canonical Wnts that converges on Runx2 (Figure 2).

In 293T cells, *tbx5*, a critical T-box gene involved in human Holt–Oram syndrome and also implicated in osteogenesis, was shown to interact directly with the chromatin coregulator TAZ (transcriptional coactivator with PDZ-binding motif), resulting in enhanced Tbx-5 activation of the osteogenic *FGF10* target gene. By recruiting HATs, TAZ mediates the opening of chromatin, thereby increasing Tbx-5 transcriptional activity [51], which may also occur during MSC osteogenesis.

The exciting new discoveries of transcriptional mechanisms driving the balance of bone formation and loss around a global osteogenic gene, *runx2*, and a specific osteogenic homeobox gene, *tbx5*, represent two strong models of transcriptional regulation of osteogenesis, and potentially other MSC lineage differentiation programs.

Adipogenesis

The nuclear hormone receptor peroxisome proliferator-activated receptor γ (PPARγ) is a critical adipogenic regulator promoting MSC adipogenesis while repressing osteogenesis [52]. The binding of PPARγ to various ligands, including long-chain fatty acids and thiazolidinedione compounds, induces the transactivation and transrepression of PPARγ. The bipotent coregulator TAZ was recently discovered to function as a coactivator of Runx2 and as a corepressor of PPARγ, thus promoting osteogenesis while blocking adipogenesis [53]. Mechanistically, the converse, in which a coactivator of adipogenic genes corepresses osteogenic genes, is also possible.

This type of cellular efficiency is plausible, given that both lineages may be derived from a common MSC.

Interestingly, another example of interplay between transcriptional cofactors of adipogenesis involves stretch-related mechano-induction. Mouse embryonic lung mesenchymal cells form myocytes under stretch induction but form adipocytes if uninduced. Stretch/non-stretch mechano-stimulation activates specific isoforms of tension-induced/-inhibited proteins (TIPs) [54], chromatin-modifying proteins with intrinsic HAT activity that have other distinctive domains such as nuclear receptor-interacting motifs. TIP-1 is expressed under non-stretch conditions and promotes adipogenesis, whereas TIP-3 promotes myogenesis. TIP-1 also provides a potential mechanistic endpoint for cytoplasmic RhoA-mediated induction of adipogenesis; that is, round formation of cells, associated with lack of cell tension, induces RhoA signaling, which promotes adipogenesis [55]. Together, these findings suggest a molecular model that potentially links mechanical induction, cell morphology, cytoskeletal signaling, and transcriptional response in the induction of MSC adipogenesis.

Myogenesis

Most investigations of myogenesis in adult stem cells are based on a small population of skeletal muscle-derived stem cells, or satellite cells. A recent study showed the highly successful induction of myogenesis from adult stromal MSCs, after transfection with activated Notch 1 [56]; however, the mechanisms of action remain unknown. Other investigations, largely focused on cardiomyogenesis, showed the importance of cell–cell contact in stimulating cardiomyogenesis by using co-cultured MSCs and cardiomyocytes, and the stimulation of MSC cardiomyogenesis in a rat intramyocardial infarct model by Jagged 1, a Notch ligand [57]. Other animal cardiac and vascular injury models and human clinical trials are being actively investigated to explore the potential regeneration of cardiac tissue.

Tenogenesis

GDF proteins, members of the TGF-β superfamily, promote the formation of tendons *in vivo* [58]. In addition to culture medium specifications, differentiation of MSCs into tenocytes *in vitro* requires mechanical loading [59], which is critical to tendon fiber alignment during development. The identity of specific differentiation gene markers to track the tenogenesis of MSCs remains unknown. Expression of *scleraxis*, which encodes a bHLH transcription factor, is detectable *in vivo* in a somitic tendon progenitor compartment, and remains expressed through mature tendon development. However, other mesenchymal tissues destined to form axial skeleton, chondrocytes [60], and ligament [61] are also *scleraxis*-positive, indicating the need for additional, more discriminating markers to follow tenogenesis. Recently, it was shown that R-Smad8 specifically transduced BMP-2 signaling in murine C3H10T1/2 cells to form tenocytes rather than osteoblasts [62]. The activation domain of R-Smad8 may be uniquely regulated or used to form distinct transcriptional complexes specific for tenogenic differentiation.

MSC niche

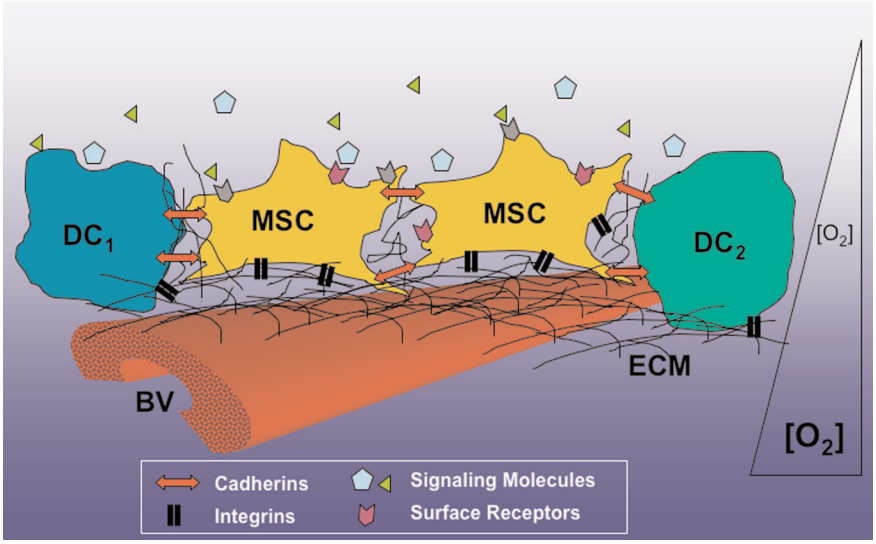
In analyzing the differentiation of stem cells, it is critical to consider the influence of their tissue of origin. MSCs are now routinely isolated from the bone marrow of many mammalian model organisms, as well as from other tissues of mesodermal origin such as adipose, muscle, bone, and tendon. Recently, multipotent cells have also been isolated from many other tissue types of non-mesodermal origin. Specifically, a recent study reported plastic-adherent MSC-like colonies derived from the brain, spleen, liver, kidney, lung, bone marrow, muscle, thymus, and pancreas of mice [63], all with similar morphologies and immunophenotypes after several passages. In another study, murine MSCs were obtained from freshly isolated cells of the heart, liver, kidney, thymus, ovary, dermis, and lung on the basis of a $CD45^-/CD31^-/Sca\text{-}1^+/Thy\text{-}1^+$ phenotype [64], raising the question of what the common *in vivo* microenvironment of the MSC might be. Is there an MSC niche that is common to all of these tissues, or do MSCs function autonomously, in a manner that is independent of their environment?

Since Schofield first introduced the concept of a stem cell 'niche' in 1978 [65], the idea has gained wide support, particularly in recent years. In brief, the niche encompasses all of the elements immediately surrounding the stem cells when they are in their naïve state, including the non-stem cells that might be in direct contact with them as well as ECM and soluble molecules found in that locale. All of these act together to maintain the stem cells in their undifferentiated state. It is then assumed that certain cues must find their way into the niche to signal to the stem cells that their differentiation potential is needed for the regeneration or repopulation of a tissue.

Cellular components

Two recent studies suggested a perivascular nature of the MSC niche (Figure 3), on the basis of the expression of α-smooth muscle actin (αSMA) in MSCs isolated from all tissue types tested [63] and the immunohistochemical localization of $CD45^-/CD31^-/Sca\text{-}1^+/Thy\text{-}1^+$ cells to perivascular sites [64]. In support of this, MSCs were found, with the use of the markers Stro-1 and CD146, lining blood vessels in human bone marrow and dental pulp [66]. These cells also expressed αSMA and some even expressed 3G5, a pericyte-associated cell-surface marker. Some researchers have hypothesized that pericytes are in fact MSCs, because they can differentiate into osteoblasts, chondrocytes, and adipocytes [67]. Localization of MSCs to perivascular niches throughout the body gives them easy access to all tissues and lends credence to the notion that MSCs are integral to the healing of many different tissues (see the 'Homing and wound healing' section below). Experiments *in vivo* that

Figure 3



Mesenchymal stem cell niche. Mesenchymal stem cells (MSCs) are shown in their putative perivascular niche (BV, blood vessel), interacting with (1) various other differentiated cells (DC_1, DC_2, etc.) by means of cell-adhesion molecules, such as cadherins, (2) extracellular matrix (ECM) deposited by the niche cells mediated by integrin receptors, and (3) signaling molecules, which may include autocrine, paracrine, and endocrine factors. Another variable is O_2 tension, with hypoxia associated with MSCs in the bone marrow niche.

perturb this perivascular environment are needed to validate this theory.

The transmembrane cell adhesion proteins, cadherins, function in cell–cell adhesion, migration, differentiation, and polarity, including in MSCs [44], and are known to interact with Wnts, which are important in MSC biology, as described above. They are also implicated in the biology of other stem cell niches [68]. Their role in the MSC niche is an unexplored territory and is crucial to an understanding of the molecular basis of the interactions between the MSC and its neighbors.

Soluble components

That the bone marrow milieu is hypoxic in nature is of particular relevance. Comparison of human MSCs cultured in hypoxic versus normoxic conditions (2% and 20% oxygen) showed that their proliferative capacity was better maintained in the former [69]. In addition, hypoxia at least doubled the number of CFU-Fs present while enhancing the expression of *oct-4* and *rex-1*, genes expressed by embryonic stem cells and thought to be pivotal in maintaining 'stemness'. These data suggest that hypoxia enhances not only the proliferative capacity but also the plasticity of MSCs. The mechanism of action of hypoxia on MSCs is currently unknown, although *oct-4* upregulation by the transcription factor HIF-2α (hypoxia-induced factor-2α) is possible [70].

The role of secreted proteins in the MSC niche is not understood. Many studies have used conditioned media and Transwell set-ups to analyze the effects of proteins secreted by various cell types on MSCs without direct cellular contact (see, for example, [71,72]). So far, we know of no studies that identify the effective proteins or that present a cell type whose secreted factors exhibit a 'niche effect' on MSCs. In other words, the cell types studied have either had no effect on MSCs or they have induced differentiation instead. Finding one or more soluble proteins that inhibit MSC differentiation while allowing proliferation would be ideal for mimicking the niche and expanding MSCs *ex vivo*.

Extracellular matrix components

Again, no specific matrix components have been identified that help to maintain MSCs in their naïve state, as a niche matrix would do. However, there is evidence that ECM alone can regulate MSC differentiation, with potential applications for tissue engineering. For example, ECM left by osteoblasts on titanium scaffolds after decellularization increased osteogenesis markers, such as alkaline phosphatase and calcium deposition, in MSCs [73]. Our recent observations also suggest that ECM deposited by microvascular endothelial cells enhances MSC endotheliogenesis (T Lozito and RS Tuan, unpublished data). Designing artificial matrices that can mimic the tissue microenvironment *in vivo* and regulate the appropriate differentiation of stem cells is a promising approach to therapeutic applications. Molecular information on ECM–MSC interactions, most probably involving integrins, which have already been implicated in niche biology in other systems (see, for example, [74]), is clearly needed.

Homing and wound healing

Another stem cell niche-related phenomenon is the homing of stem cells to sites of injury and subsequent wound healing. Although some tissue repair may be accomplished by the division of indigenous differentiated cells, such cells are most frequently post-mitotic. Thus, signaling to progenitor/stem cells to home to the site of injury and differentiate into the required cell type is required. To understand the niche, it is important to analyze not only what keeps stem cells in their niche but also what signals them to emigrate from it.

Even in healthy animals, MSCs are capable of homing to tissues other than the bone marrow, such as lung and muscles [75]. Interestingly, the capacity of an MSC for homing seems to be related in part to its expression of Stro-1 (see the 'MSC markers' section above) [7]. Whereas Stro-1-negative cells were better able to aid in the engraftment and survival of HSCs, Stro-1-positive cells were more capable of homing and engrafting to most of the tissues studied. Exciting new work *in vitro* shows that MSC migration is regulated by stromal-derived factor-1/CXCR4 and hepatocyte growth factor/c-Met complexes, and involves matrix metalloproteinases [76]. *In vivo* expression profiles of the responsible factors will shed light on when, where, and how MSCs migrate. What is known is that injury alters the patterns of migration and differentiation of exogenously added MSCs. In the mouse, irradiation of both the whole animal and specific sites caused injected MSCs to engraft to more organs and in higher numbers than in unconditioned mice [75].

In addition, it seems that mature cells that have been injured are able to secrete not only homing signals but also differentiation signals. Rat bone marrow-derived MSCs, for example, begin myogenesis in response to conditioned medium from damaged but not undamaged skeletal muscle [77]. Other studies *in vitro* suggest that some uninjured cells can also induce differentiation when direct contact is allowed. Our preliminary results show that direct co-culturing with osteoblasts enhances the osteogenesis of MSCs (CM Kolf, L Song and RS Tuan, unpublished data). Liver cells also seem to be capable of inducing hepatogenesis [78]. However, it is important to note that mature cells do not always induce MSC differentiation along their own lineage. Direct contact with chondrocytes induces osteogenesis but not chondrogenesis [72]. Clearly, the environment of an MSC is a critical defining factor of its identity.

Conclusion

Adult MSCs are a potentially powerful candidate cell type for regenerative medicine as well as for the study of cellular differentiation. A key requirement for both fields is the identification of MSCs *in vivo*. In mouse, genetic markers and pulse–chase techniques can be used to label stem cells [79]. In other systems, asymmetric division has been shown to be integral to stem cell self-renewal. This unique property of stem cells has been exploited to identify mouse muscle satellite cells [80] and could possibly be used to identify MSCs *in vivo* and to study their division. Once the true MSC population is identified, global characterization using gene arrays and surface antigen profiling may be achieved. The roles of each component of the MSC system should then be functionally analyzed. Critical challenges include identifying the signaling factors that promote the self-renewal of MSCs, as well as elucidating the master transcriptional regulatory switches and the crosstalk between the signaling pathways that mediate exclusive lineage differentiation in MSCs. Future investigations should incorporate combinatorial knockdown approaches using inducible and stable expression systems to address redundancy in signaling functions, for example within the TGF-β and Wnt families. The identification of specific cell-surface receptors activated by signaling molecules, such as TGF-βs (BMPs) and Wnts, during self-renewal and cytodifferentiation is also crucial to understanding the link between extracellular and intracellular signaling networks. Finally, alterations in the MSC niche will help to determine the intrinsic and extrinsic specificity of MSC regulators. In an elegant model experiment, quiescent muscle and liver stem cells of aged mice were rejuvenated when exposed to the circulating blood of younger animals [81]. That an extrinsic change can enhance stem cell functions presents hope for harnessing the healing powers of adult stem cells in the future.

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Competing interests

The authors declare that they have no competing interests.

Acknowledgment

This work was supported by the Intramural Research Program of NIAMS, NIH (Z01 AR41131).

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