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Trafficking and Differentiation of Mesenchymal Stem Cells

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ABSTRACT

Mesenchymal stem cells (MSCs) are a heterogeneous population of stem/progenitor cells with pluripotent capacity to differentiate into mesodermal and non-mesodermal cell lineages, including osteocytes, adipocytes, chondrocytes, myocytes, cardiomyocytes, fibroblasts, myofibroblasts, epithelial cells, and neurons. MSCs reside primarily in the bone marrow, but also exist in other sites such as adipose tissue, peripheral blood, cord blood, liver, and fetal tissues. When stimulated by specific signals, these cells can be released from their niche in the bone marrow into circulation and recruited to the target tissues where they undergo in situ differentiation and contribute to tissue regeneration and homeostasis. Several characteristics of MSCs, such as the potential to differentiate into multiple lineages and the ability to be expanded ex vivo while retaining their original lineage differentiation commitment, make these cells very interesting targets for potential therapeutic use in regenerative medicine and tissue engineering. The feasibility for transplantation of primary or engineered MSCs as cell-based therapy has been demonstrated. In this review, we summarize the current knowledge on the signals that control trafficking and differentiation of MSCs. J. Cell. Biochem. 106: 984–991, 2009.

KEY WORDS: MESENCHYMAL STEM CELLS; CELL DIFFERENTIATION; CELL TRAFFICK

BASIC BIOLOGY OF MSCs

Adult MSCs were first discovered by Friedenstein et al. [1966] more than 40 years ago. They were described as bone marrow-derived clonogenic, plastic-adherent cells capable of differentiating into osteoblasts, adipocytes, and chondrocytes. Thereafter, various research groups reported different populations or subsets of bone marrow stromal cells which share similar but not identical features with the originally described MSCs. These other cells are referred to as bone marrow stromal stem cells (BMSSC) [Gronthos et al., 2003], mesenchymal stem cells/marrow stromal cells (MSC) [Caplan, 1994; Lazarus et al., 1995], marrow-isolated adult multipotent inducible cells (MIAMI) [D'Ippolito et al., 2004], multipotent adult progenitor cells (MAPC), and mesenchymal adult stem cells (MASCS) [Belema-Bedada et al., 2008]. These cells are either MSC-like cells, or contain partially overlapping population of MSCs. MSCs, in general, display significant heterogeneity. There is, thus far, no uniformly accepted clear and specific definitive phenotype or surface markers for the prospective isolation of MSCs. Instead, MSCs are defined retrospectively by a constellation of characteristics in vitro, including a combination of phenotypic markers and multipotent differentiation and functional properties. The minimal requirement for a population of cells to qualify as MSCs, as suggested by the International Society for Cytotherapy, is to meet the three criteria, including (i) the plastic adherence of the isolated cells in culture, (ii) the expression of CD105, CD73, and CD90 in greater than 95% of the culture, and their lack of expression of markers including CD34, CD45, CD14 or CD11b, CD79α or CD19 and HLA-DR in greater than 95% of the culture, and (iii) the differentiation of the MSCs into bone, fat and cartilage [Dominici et al., 2006].

Though originally isolated from bone marrow, MSCs and MSC-like cells have been found to be harbored in various other sites, including adipose tissue, periosteum, synovial membrane, synovial fluid (SF), skeletal muscle, dermis, deciduous teeth, pericytes, trabecular bone, infrapatellar fat pad, articular cartilage, umbilical cord blood, placenta, liver, spleen, and thymus [Chen et al., 2006; Bianco et al., 2008; Mimeault and Batra, 2008]. For example, MSCs can be isolated from adipose stromovascular fraction and may reside in a perivascular location and express CD34 and smooth muscle actin [Lin et al., 2008]. These adipose-derived stem cells can renew themselves for many passages in culture [Zuk et al., 2002] and be induced to differentiate into adipose tissue, muscle, bone, cartilage, endothelium, and even neuronal cells, similar to bone marrow-derived mesenchymal stem cells [Jiang et al., 2002]. Some studies

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have suggested that the adipocyte precursor is a circulating cell derived from bone marrow, although this is controversial. Both stromovascular fraction-derived stem cells and bone marrow-derived mesenchymal stem cells share some cell surface markers, such as CD105 [Crisan et al., 2008]. Parenthetically, although most pericytes, adipocytes, myoblasts, and osteoblasts have been suggested to be of mesodermal origin, in all four cases, similar cell types in the head and neck appear to differentiate from neural crest, which is derived from embryonic neuroectoderm [Billon et al., 2007; O'Rahilly and Muller, 2007]. Indeed, it has been suggested that cells with mesenchymal stem characteristics reside in virtually all postnatal organs and tissues. However, the relationship between traditional bone marrow-derived MSCs and these other MSC-like populations remains to be fully clarified. Moreover, MSC-like cells can be isolated from pathological tissues such as the rheumatoid arthritic joint, and these cells express bone morphogenetic protein receptors [Marinova-Mutafchieva et al., 2000].

MSCs isolated from different tissues show similar phenotypic characteristics. However, they show different propensities to proliferate and differentiate in response to stimulation with various growth factors. Therefore, it is not clear whether or not these are the same MSCs. A study that compared human MSCs derived from bone marrow, periosteum, synovium, skeletal muscle, and adipose tissue revealed that synovium-derived MSCs exhibited the highest capacity for chondrogenesis, followed by bone marrow-derived and periosteum-derived MSCs [Sakaguchi et al., 2005]. Isolation methods, culture surface, culture medium, seeding density, as well as treatment with various growth factors and chemicals influence the expansion, differentiation, and immunogenic properties of MSCs [Sotiropoulou et al., 2006]. In addition, donor age and disease stage can also influence MSC yield, proliferation rate, and differentiation potential.

Different tissue-specific stem cells vary in phenotype, morphology, proliferation potential and differentiation capacity, but display many common characteristics attributed to their bone marrow counterparts, suggesting that MSC-like populations share a similar ontogeny. Emerging evidence suggests that the perivascular niche is a common stem cell microenvironment for resident MSC-like populations within the different tissues [Bianco et al., 2001; Gronthos et al., 2003; Shi and Gronthos, 2003; Djouad et al., 2007; Sacchetti et al., 2007; Zannettino et al., 2008]. Recently, it has been reported that MSCs isolated from multiple adult and fetal organs as well as pericytes that reside in close proximity within the vascular wall are positive for the perivascular marker CD146 and display similar properties, such as the capacity for differentiation towards adipogenic, osteogenic, and chondrogenic cell lineages [Covas et al., 2008]. This is in accord with previous studies demonstrating that CD146 identifies a minor fraction of bone marrow, adipose tissue and dental pulp tissue-derived MSCs that express stromal precursor antigen-1 (STRO-1), which co-localizes with the pericyte markers 3G5 and α-smooth muscle actin, in situ [Filshie et al., 1998; Gronthos et al., 2003; Shi and Gronthos, 2003; Schwab et al., 2008; Zannettino et al., 2008]. Furthermore, studies by Canfield and colleagues have proposed that STRO-1^+ pericytes may in fact be the source of MSC-like cells identified in different tissues [Doherty et al., 1998; Farrington-Rock et al., 2004]. This notion raises the intriguing possibility that underlying stromal elements associated with the vasculature in different organs may possess the potential for local tissue regeneration while supporting the maintenance and regulation of local parenchymal tissues via support of the tissue's vasculature. A very recent study has demonstrated that adipocytes originate from precursor cells that indeed reside within the walls of the blood vessels that support fat tissue [Kahn, 2008; Tang et al., 2008].

REGULATORY SIGNALS FOR MULTIPLE-LINEAGE DIFFERENTIATION OF MSCs

MSCs are characterized by their intrinsic self-renewal capacity, which is reflected in its clonogenic property and multi-lineage differentiation potential (Fig. 1). Although not immortal, they have the ability to expand numerous times in culture while retaining their growth and pluripotent potential. In addition to their capacity to differentiate into chondrocytes, osteoblasts, and adipocytes, MSCs may serve as hematopoiesis-supporting stromal cells [Prockop, 1997; Pittenger et al., 1999]. Although controversial, MSCs have also been reported to differentiate into myocytes and cardiomyocytes and even into cells of non-mesodermal origin, including hepatocytes, insulin-producing cells, and neurons [Jiang et al., 2002]. While many studies have looked into the pluripotent differentiation potential of MSCs and broad overall knowledge has been accumulated regarding this subject, the specific mechanisms responsible for these versatile cells' broad differentiation capacity are poorly understood.

The specific lineage commitment of MSCs is largely influenced by culture condition, especially growth factors, in vitro. Table I summarizes the biological and chemical signals which determine the multilineage differentiation of MSCs. Growth factors that have regulatory effects on MSCs include members of the transforming growth factor-beta (TGF-β) superfamily, the insulin-like growth factors (IGF) [Matsuda et al., 2005], the fibroblast growth factors (FGF) [Ito et al., 2008], the epidermal growth factor (EGF) [Kratchmarova et al., 2005], the platelet-derived growth factor (PDGF), the vascular endothelial growth factor (VEGF) [Liu et al., 2007], and the family of growth factors known as Wnt.

With respect to promoting chondrogenesis, the most potent inducers are the TGF-β family, including TGF-β1, TGF-β2, and TGF-β3, as well as bone morphogenetic proteins (BMPs). For human MSCs, TGF-β2, and TGF-β3 were shown to be more active than TGF-β1 in promoting chondrogenesis and although cellular content is similar after culture, significantly more proteoglycans and collagen type II can be produced after stimulation with the former two inducers [Barry et al., 2001]. BMPs, known for their involvement in cartilage formation, can act alone or in concert with other growth factors to induce or enhance MSC chondrogenic differentiation. For example, BMP-2, BMP-4, or BMP-6, combined with TGF-β3, induced chondrogenic phenotype in cultured human bone marrow-derived MSC pellets, with BMP-2 having the most pronounced effect [Sekiya et al., 2005]. Adipose tissue-derived MSCs lack of expression of TGF-β type I receptor and have reduced expression of BMP-2, BMP-4, and BMP-6 when compared with bone marrow MSCs.

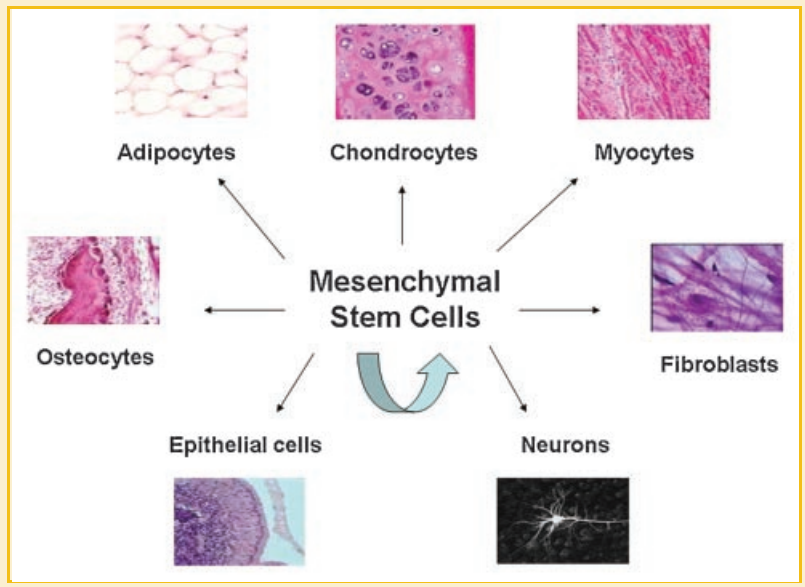

Fig. 1. Pluripotent capacity of MSC to differentiate into mesodermal and non-mesodermal cell lineages, including osteocytes, adipocytes, chondrocytes, myocytes, fibroblasts, epithelial cells, and neurons (solid arrows). In addition, MSCs are capable to self-renewal (block arrow).

Therefore, supplementation with BMP-6 and TGF-β is necessary for their chondrogenic differentiation, with BMP-6 being the stronger stimulator for chondrogenic differentiation [Hennig et al., 2007]. Polymorphism and altered gene expression in the Wnt signaling pathway have recently been associated with rheumatoid arthritis and osteoarthritis [Sen, 2005]. Canonical Wnt signaling in coordination with TGF-β and BMP signaling has been shown to enhance MSC differentiation [Tuli et al., 2003; Zhou et al., 2004]. In addition, canonical and noncanonical Wnts have been shown to cross talk with each other in regulating stem cell proliferation and osteogenic differentiation [Baksh and Tuan, 2007].

The regulatory signals of growth factors are delivered through specific signal transduction pathways that control downstream transcription factors. With respect to growth factor-induced chondrogenic differentiation, it is known that various intracellular signaling pathways, such as mitogen-activated protein kinases (MAPK) and Smads, are activated, which induce several specific transcription factors (sox9, sox5, and sox6). This in turn results in the production of extracellular matrix (ECM) proteins, including collagen type II, aggrecan, and cartilage oligomeric matrix protein, which are required for cartilage formation. Sox9 is one of the most important molecules for the expression of the cartilaginous phenotype. It has been demonstrated that expression of exogenous sox9 in bone marrow-derived MSCs leads to increased proteoglycan deposition [Tsuchiya et al., 2003]. Sox9 is now considered a "master switch" in chondrogenesis.

In addition to growth factors, other chemicals play critical roles in determining the lineage commitment of MSCs. For instance, incubation of MSCs with dexamethasone, insulin, isobutyl methyl xanthine, and indomethacin can preferentially promote their differentiation towards adipogenic lineage. Lipid-rich vacuoles accumulate within cells, and they express peroxisome proliferation-activated receptor γ2, lipoprotein lipase, and the fatty acid-binding protein aP2 [Pittenger et al., 1999]. The lipid vacuoles eventually combine and fill the cells. Accumulation of lipid in these vacuoles is assayed histologically by oil red O staining. It has also

TABLE I. Regulatory Signals for Multiple-Lineage Differentiation of MSCs

	Multilineage differentiation potential	Representative Refs.
Biological signals		
TGF-β	Chondrogenic	Barry et al. [2001], Sekiya et al. [2005]
IGF-1	Chondrogenic,	Matsuda et al. [2005]
bFGF	Chondrogenic, Osteogenic, neural	Ito et al. [2008]
EGF	Chondrogenic	Kratchmarova et al. [2005]
PDGF	Chondrogenic, myofibroblastic	Kratchmarova et al. [2005], Nedeau et al. [2008]
VEGF	Endothelial	Liu et al. [2007]
Wnt	Chondrogenic, osteogenic, neural	Baksh and Tuan [2007]
Chemical signals		
Dexamethasone + isobutyl methyl xanthine + indomethacin	Adipogenic	Pittenger et al. [1999]
5-azacytidine + amphotericin B	Myoblastic	Wakitani et al. [1995]
Nicotinamide + 2-ME	Islet β-cell-like	Chen et al. [2004]
2-ME DMSO	Neural	Kohyama et al. [2001], Woodbury et al. [2000]
Retinoic acid		Mareschi et al. [2006]

been reported that, when treated with 5-azacytidine and amphotericin B, MSCs differentiate into myoblasts that fuse into rhythmically beating myotubes [Wakitani et al., 1995]. Supplyment with nicotinamide and beta-mercaptoethanol [2-ME] induces differentiation of rat MSCs into islet β-cells [Chen et al., 2004]. Furthermore, by relatively simple chemical means (treatment with 2-ME or dimethylsulfoxide [DMSO] and butylated hydroxyanisol [BHA]), MSCs can be induced to differentiate into neuron-like cells [Woodbury et al., 2000; Kohyama et al., 2001]. Addition of retinoic acid facilitates neurallike differentiation [Mareschi et al., 2006]. However, in contrast to differentiated cells, which express typical neuronal cell surface makers, these neuron-like cells lack the voltage-gated ion channels necessary for generation of action potentials; therefore, these cells may not actually be classified as true neurons [Hofstetter et al., 2002].

While we have come to understand a number of pathways that mediate growth factor-induced signal transduction in MSC differentiation, the molecular mechanisms underlying chemical-induced lineage specification of MSCs remain mostly unknown. Moreover, little is known about the in vivo mechanisms underlying these chemical-induced regulatory signals since many of those substances tested in vitro do not exist naturally within humans or experimental animals. However, increased understanding of these molecular cascades remains of paramount importance since it may yield new opportunities for understanding some environmental diseases and/or developing new pharmacologic therapeutic agents.

SIGNALS THAT CONTROL TRAFFICKING OF MSCs

Cell-based therapy is a promising therapeutic option for a variety of diseases. The efficiency of this therapy to augment recovery from damaged tissues depends on not only sufficient amount of MSCs, but also efficient delivery of these cells to the desired target tissue. Recruitment of bone marrow-derived MSCs to repair damaged tissues and regeneration of the tissue is a complex multi-step process. It involves sensing the signal from the remote injured tissue that calls for the release of MSCs from their storage niche into circulation, homing of circulating MSCs to the target tissues, and in situ proliferation and differentiation of MSCs into matured, functional cells (Fig. 2).

The release of MSCs from their niche in the bone marrow into circulation is known as mobilization. The molecular mechanisms for mobilization of MSCs are poorly understood. Knowledge about the nature of signals released from the injured tissue to mobilize MSCs in the bone marrow is also very limited. One hypothesis is that cytokines and/or chemokines that are upregulated under injury conditions are released into circulation from remote tissues, stimulating MSCs to down-regulate the adhesion molecules that hold them at their niche. Cytokines and chemokines play critical roles in regulating mobilization, trafficking, and homing of stem/progenitor cells. Many of these factors are chemoattractants. Stromal cell-derived factor (SDF)-1α and its receptor, CXCR4, appear to play an important role in modulating mobilization of MSCs. For example, over-expression of CXCR4 on MSCs augments myoangiogenesis in the infarcted myocardium [Zhang et al., 2008]. Similarly, over-expression of IGF-1 in MSCs induces massive stem cell mobilization via SDF-1α signaling and culminates in extensive myoangiogenesis in the infarcted heart [Haider et al., 2008]. Interestingly, the SDF-1α/CXCR4 axis has also been known to be critical for mobilization of other progenitor/stem cells, such as the endothelial progenitor cells (EPCs) [Gallagher et al., 2007; Liu and Velazquez, 2008]. Inhibition of the SDF-1α/CXCR4 interaction partially blocks the homing of progenitor/stem cells to the ischemic myocardium [Abbott et al., 2004]. Likewise, suppression

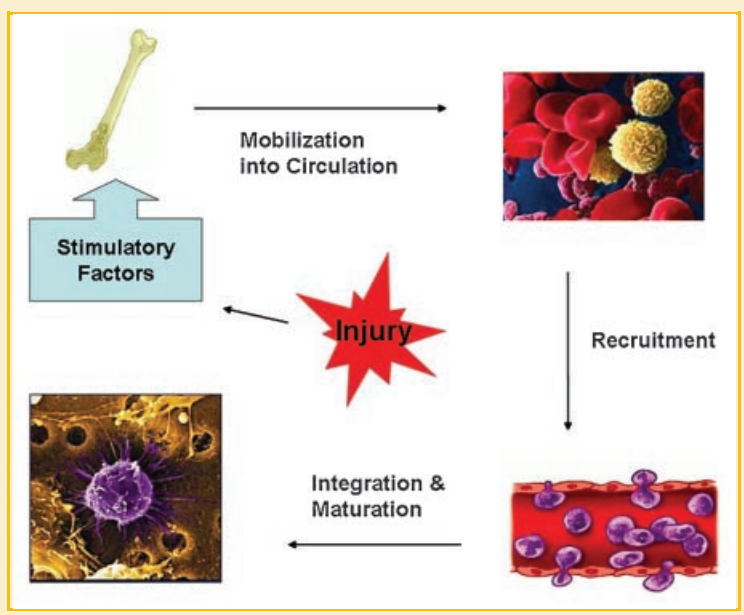

Fig. 2. Injury in the periphery releases stimulatory factors that cause mobilization of MSCs from bone marrow into the circulation. At the site of injury, certain molecules expressed on the endothelium causes recruitment of MSCs, where they transmigrate from blood vessels and undergo in situ maturation and integrate into the injured tissue to bring about healing.

of CXCR4 by anti-CXCR4 neutralizing antibodies significantly reduces SDF-1α-induced adhesion of EPCs to mature endothelial cell monolayers, the migration of EPC in vitro [Ceradini et al., 2004], and the in vivo homing of myeloid EPC to the ischemic limb in the hind limb ischemia model [Walter et al., 2005]. It could be portulated that similar mechanisms are utilized in the mobilization of several populations of stem/progenitor cells from the bone marrow.

In addition to SDF-1α, high mobility group box-1 (HMGB-1) is known to act as a chemoattractant for inflammatory cells, stem cells, and EPC in vitro and in vivo [Palumbo and Bianchi, 2004; Chavakis et al., 2007]. HMGB-1 is a nuclear protein, which is released extracellularly upon activation of cells by inflammatory cytokines and during cell necrosis.

Recently, we have investigated the role of platelet-derived growth factor-B (PDGF-B)-activated fibroblasts in regulating recruitment, migration and differentiation of murine bone marrow MSCs in an in vitro wound healing assay and a novel three-dimensional (3D) model, since local resting resident fibroblasts are activated after injury and play a critical role in recruiting MSCs. PDGF-B-activated fibroblasts caused significant increases in MSC migration velocity compared to control as demonstrated by time-lapse photography in wound healing assay. Consistently, invasion/migration of MSCs into 3D collagen gels was enhanced in the presence of PDGF-B-activated fibroblasts. In addition, PDGF-B-aFBs induced differentiation of MSCs into myofibroblast. The regulatory effects of PDGF-B-activated fibroblasts are likely to be mediated by basic fibroblast growth factor (bFGF) and epithelial neutrophil activating peptide-78 (ENA-78 or CXCL5) as protein array analysis indicated elevated levels of these two soluble factors in culture supernatant of PDGF-B-activated fibroblasts. Blocking antibodies against bFGF and CXCL5 were able to inhibit both trafficking and differentiation of MSCs into 3D collagen gels while supplement of exogenous bFGF and/or CXCL5 promoted invasion/migration of MSCs into 3D collagen gels [Nedeau et al., 2008]. Our results reveal that PDGF-B-activated fibroblasts play a key role in the recruitment/migration and differentiation of MSCs and implicate a bFGF- and CXCL5-dependent mechanism in mediating these effects.

Homing mechanisms of MSCs to the sites of target tissues involve a cascade of events, including rolling of MSCs in the blood vessels, adhesion onto the endothelial cell surface lining the capillaries, transendothelial migration, extravasation from the blood vessels and migration through the extracellular matrix into the target injured area. Recent studies support the idea that MSCs may utilize adhesion molecules for homing to sites of damaged tissues similar to the adhesion molecules engaged by leukocytes for recruitment to sites of inflammation [Luster et al., 2005; Ley et al., 2007]. In fact, many of the molecules known to be involved in the tethering, rolling, adhesion, and transmigration of leukocytes from the bloodstream into tissues are known to be expressed on MSCs. These molecules include integrins, selectins, CAMs, and chemokine receptors.

The process of rolling is essential for cell homing since it selectively slows down subsets of MSCs, allowing their subsequent endothelial adhesion and transmigration under conditions of physiological shear force. P-selectin and its counterligand have been suggested to be involved in the rolling of MSCs and adhesion to endothelial cells. Using intravital microscopy, Ruster et al. [2006] observed that intravenously administered human MSCs can roll along the walls of the blood vessels in the ear veins of mice, and this phenomenon was significantly decreased in P-selectin$^{-/-}$ mice. Moreover, in an in vitro assay, human MSCs rolling upon human umbilical vein endothelial cells under shear flow conditions were significantly reduced in the presence of a neutralizing P-selectin antibody. As neither P-selectin glycoprotein ligand-1 nor the alternative ligand CD24 was present on human MSCs, it was proposed that a novel MSC-expressed carbohydrate ligand was the counterligand for P-selectin expressed on endothelial cells. Thus, MSCs, like leukocytes, roll upon endothelial cells as the first stage in their recruitment to the needed repair areas.

Other adhesion molecules mediating MSC-endothelial cell interaction include various integrins and CAMs, such as α1, α2, α3, α4, α5, αv, β1, β3, β4, VCAM-1, ICAM-1, ICAM-3, ALCAM, and endoglin/CD105 [Minguell et al., 2001; Krampera et al., 2006]. These adhesion molecules and their counterligands are expressed on either MSCs or endothelial cells. Approximately half of human MSCs express the integrin very late antigen (VLA)-4 (α4β1, CD49d). In vitro studies demonstrate that, under conditions of shear flow, binding of human MSCs to endothelial cells is mediated by VLA-4 [Ruster et al., 2006]. Treating endothelial cells with a neutralizing antibody to its counterpart adhesion molecule VCAM-1 induces a similar decrease in human MSC adherence. Thus, the VLA-4/VCAM-1 axis is responsible for mediating firm adhesion of human MSC to endothelial cells.

Recently, Sackstein and colleagues have demonstrated a novel function of CD44 as a homing molecule for bone marrow-derived MSCs. They found that a distinct glycoform of CD44 mediates trafficking of human MSCs to bone through interactions with E-selectin, which is constitutively present on marrow vasculature [Sackstein et al., 2008].

CD44 adhesion molecules represent a large family of transmembrane glycoproteins. The ligand-binding affinity of CD44 depends on its glycosylation pattern [Katoh et al., 1995]. Sackstein et al. [2008] created a stereospecific fucosylation of CD44 and observed that such glycoform of CD44 confers robust rolling of MSCs on vascular E-selectin in vitro and induces significantly increased homing of intravenously injected human MSCs to bone in NOD/SCID mice. While CD44 has multiple ligands, this receptor/ligand specificity of CD44—engineered in vitro—may offer an opportunity to direct the migration of stem cells to one organ versus another. If cell surface molecules expressed on organ-specific endothelium contribute to stem cell-endothelial cell adhesive interactions, the manipulation of these ''homing signatures'' may allow a more precise targeting of stem cells, directing them to the damaged areas to the exclusion of undesired organs.

It should be pointed out that, although it would seem likely that MSCs employ some of the same molecules as that of leukocytes for transmigration into tissues, specific differences in the use of adhesion molecules may also exist between these two cell types. For example, both L- and E-selectin are known to be involved in the initial rolling stage on leukocytes, however, expression of L-selectin is low or absent on the surface of MSCs, and the role of E-selectin has

not yet been determined [Ruster et al., 2006]. Another difference is that PECAM-1/CD31, which plays an important role in leukocyte transmigration across the endothelium, is not expressed on MSCs.

It is unclear whether bone marrow-derived circulating MSCs and tissue-derived MSCs utilize identical trafficking mechanisms. A comparison between the cell adhesion molecule expression profiles of these distinct MSC populations may provide further insight into the potential mechanisms of MSC homing.

The Rho family of GTPases are key regulators of actin cytoskeletal dynamics and affect many cellular processes, including cell migration and adhesion, thus they have been studied in regards to trafficking of MSCs. Most Rho GTPases switch between an active GTP-bound form and an inactive GDP-bound form. The cycling of Rho GTPases between these two states is regulated by three sets of proteins, guanine nucleotide-exchange factors (GEFs), GTPase-activating proteins (GAPs), and guanine nucleotide-dissociation inhibitors (GDIs). Rho proteins interact with and activate downstream effectors when bound to GTP, thereby stimulating cell migration and adhesion. Gu et al. [2003] have implicated a crucial role of the activation status of Rac GTPase in the proliferation and migration of hematopoietic stem and progenitor cells. While it has been generally believed that signaling through the Rho family of GTPases would influence the migration response of MSCs, experimental data has not been consistent. It has been demonstrated that inhibition of Rho induces rearrangement of actin cytoskeleton in MSCs and renders them susceptible to induction of migration by physiological stimuli [Jaganathan et al., 2007]. However, when investigators studied the potential involvement of Rho GTPase in controlling migration of MSCs using a 3D matrix model, they found that neither Rho nor the Rho-effector, Rho kinase (ROCK), are required for 3D migration of MSCs [Provenzano et al., 2008]. Therefore, the role of the Rho family of GTPases in regulating migration of MSCs in vivo still needs further investigation.

In clinical settings, MSCs can be therapeutically utilized via several approaches, most notably by either direct site delivery or systemic intravascular administration. In the case of the former, MSCs are delivered to the local tissue directly via local or intralesional implantation. Therefore, mobilization and homing/recruitment mechanisms are not required, but survival, differentiation and proliferation signals are still paramount to achieve success. In the local delivery setting, MSCs interact directly with the microenvironment and can be induced to undergo in situ proliferation and differentiation, and contribute to tissue repair if the proper microenvironment is provided. Alternatively, when a systemic intravascular administration approach is utilized, this bypasses the mobilization process required in the natural situation, but the homing/recruitment mechanisms are still required for MSCs to reach their target tissue. Understanding the molecular mechanisms underlying homing of both bone marrow-derived circulating MSCs and tissue-derived MSCs will help expand the potential clinical applications of MSCs.

SUMMARY

MSCs are known to possess multi-lineage differentiation potential and can be directed to grow into specific cell lineages under certain micro-environmental conditions. This characteristic makes these cells uniquely suited for various therapeutic possibilities such as supporting tissue regeneration, correcting inherited disorders, dampening chronic inflammation, and delivering biological agents. Therefore, administration of MSCs could be a promising new strategy to treat a variety of diseases. However, the mechanisms that govern MSCs to undergo specific lineage differentiation remain largely unclear. This situation makes the clinical application of stem cell-based therapy highly uncertain unless this critical knowledge is obtained. This is an area that needs tremendous research efforts hereon. Another area that is equally challenging is the mechanisms underlying each step of the trafficking of MSCs. Evidence suggests that chemokines, their receptors, and other adhesion molecules are involved. Identifying these molecules and studying their roles in regulating trafficking of MSCs may allow the development of therapeutic strategies to enhance the recruitment of bone marrow-derived and/or tissue-specific MSCs. Furthermore, understanding the survival, proliferation, and differentiation signals will further expand potential therapeutic applications of either native and ex vivo-cultured MSCs utilized to repair damaged tissues. In summary, fulfilling the promise of regenerative medicine critically depends on identifying the mechanisms and the molecules that control and mediate MSCs lineage-specific differentiation as well as tissue-specific stem cell homing in addition to the better understood cell survival and proliferation signaling cascades.

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