A SAGE View of Mesenchymal Stem Cells

Donald G. Phinney

Center for Gene Therapy, Tulane University Health Sciences Center, New Orleans, LA, USA

Mesenchymal stem cells (MSCs) were initially defined by their capacity to differentiate into connective tissue cell lineages and support hematopoiesis. More recently, MSCs have demonstrated some degree of therapeutic efficacy in a broad range of diseases including neurological and auto-immune disorders, stroke, diabetes, and chronic inflammatory conditions. An emerging paradigm suggests that MSCs alter the tissue microenvironment via paracrine signaling to induce angiogenesis, alter immune cell function, block inflammation, and stimulate growth of host cells to affect tissue repair. However, these activities appear at odds with the term “mesenchymal stem cell”, which by definition implies a rare cell population that through a process of self-renewal yields progenitors that differentiate hierarchically into connective tissue cell types to maintain tissue homeostasis. Analysis of the MSC transcriptome via serial analysis of gene expression (SAGE) revealed that populations express a diverse array of proteins that are important for mesoderm specification but that also regulate various biochemical processes important in bone and marrow, such as angiogenesis, hematopoiesis, cell communication, and neural activities. Moreover, different classes of these regulatory proteins were found to be expressed within distinct sub populations of MSCs. Therefore, MSC populations appear to be more heterogeneous than initially envisions. Evidence is provided that this functional heterogeneity contributes significantly to the therapeutic effects of MSCs.

Keywords: Mesenchymal stem cells, Marrow stromal cells, Multi-potent mesenchymal progenitor cells, Serial analysis of gene expression

The discovery of MSCs and their biological characteristics

Friedenstein and co-workers were the first to demonstrate that whole bone marrow transplanted under the kidney capsule of mice formed a heterotopic osseous tissue that was self-maintaining (not remodeled by host tissue) and functioned as a hematopoietic organ (reviewed in 1). Friedenstein also showed that heterotopic osseous tissue could be regenerated in vivo following serial transplantation of marrow cells to secondary recipients, indicating

that this activity was due to a unique stem cell in bone marrow distinct from that responsible for hematopoiesis. Subsequent studies further revealed that the osteogenic capacity of marrow segregated with the fibroblastoid cell fraction, which was enriched from marrow by its propensity to adhere to tissue culture plastic. These plastic adherent cells, which were referred to as marrow stromal cells, retained the capacity to form bone in vivo even after culture expansion in vitro.

One immediate consequence of Friedenstein's work was the use of marrow stromal cells as feeder layers to establish long-term bone marrow cultures (2, 3). This advance allowed hematopoiesis to be studied in vitro and led to the identification of various adhesion molecules, growth factors, and cytokines secreted by marrow stromal cells that regulated granulopoiesis, lymphopoiesis, and supported maintenance of the hematopoietic stem cell (4). Another was the subsequent realization that marrow stromal cells were capable of multi-lineage differentiation (5, 6), which validated in part the mesengenic process first

Accepted for publication March 30, 2009

Correspondence to **Donald G. Phinney**

Professor of Microbiology and Immunology, Associate Director of Research, Center for Gene Therapy (SL-99), Tulane University Health Sciences Center, 1430 Tulane Avenue, New Orleans, LA 70112

Tel: +504-988-7725, Fax: +504-988-7710

E-mail: dphinne@tulane.edu

proposed by Caplan (7). Studies showing that clonal populations of rodent (8) and human (9) stromal cells were multi-potent led to the adoption of the term “mesenchymal stem cell” to denote this cell population.

Despite these advances and the more recent emergence of MSCs as a prominent resource for cell-based therapy, many aspects of their biology remain indeterminate. Specifically, the term “mesenchymal stem cells” implies a homogeneous population of self-renewing stem cells that are maintained in vitro over an extended time course of culture expansion. Although most MSC populations, with the exception of those from mouse bone marrow, exhibit a high proliferative capacity and can be expanded clonally in vitro (10), empirical evidence demonstrating self-renewal via a process of asymmetric cell division is lacking. Alternatively, various groups have demonstrated that populations are functionally heterogeneous with respect to their differentiation potential. For example, analysis of a large cohort of clonally-derived human MSC populations revealed that only one in three clones retained the capacity for multi-lineage differentiation, which was subsequently diminished as a function of passage in vitro (11). Other studies have shown that fewer than 50% of human MSC clones that undergo osteogenic differentiation in vitro are capable of forming heterotopic osseous tissue in vivo (12). Analysis of a large number of mouse marrow stromal cell lines has also revealed inherent differences in the capacity of cells to support hematopoiesis (13).

More recently, a number of surface epitopes have been identified that can be used to enrich MSCs from bone marrow. For example, Pittenger et al. demonstrated that parental and clonal populations of human MSCs that exhibit the capacity for tri-lineage differentiation in vitro uniformly express the antigens CD73 and CD105 (9). However, these cells were noted to exhibit a decline in osteogenic and chondrogenic potential as a function of passage, which was not discernable by a change in surface phenotype (14). Populations with the capacity for osteogenic and adipogenic have also been enriched from bone marrow via positive selection for CD271 (15). Notably, the CD271⁺ cell fraction has also been shown to express CD34, CD133, platelet-derived growth factor receptor-beta (CD140b), HER-2/erbB2 (CD340), and frizzled-9 (CD349) (16). Perspective isolation of bone marrow cells based on selection for stage specific embryonic antigen 4 (SSEA-4) (17) or the pericyte marker CD146 (18) has also been reported to yield populations that are capable of generating heterotopic osseous tissue in vivo. When plated at clonal density, the SSEA-4⁺ cells generated a high percentage (~75%) of clones that exhibit tri-lineage potential in vitro

(18). Recently we analyzed the differentiation potential of human clones derived from parental populations that meet the recently established criteria for MSCs (19), and found that only about one in three clones were capable of tri-lineage differentiation ([K. O'Connor], unpublished data, 2009). Therefore, the term “mesenchymal stem cell” does not appear to accurately characterize populations utilized in most laboratories since they exhibit varying degrees of functional heterogeneity with respect to their differentiation potential.

SAGE and the MSC transcriptome

To better define the molecular phenotype of MSCs, my laboratory catalogued the transcriptome of human and murine populations via SAGE (Table 1). Initially, we sequenced 17,767 tags from a single cell-derived colony of human MSCs cultured under conditions that selected for high colony-forming efficiency and multi-potency (10), and subsequently sequenced 69,937 tags from mono-layer cultures (non-clonal) derived from the same donor. We also sequenced 59,007 tags from a population of primary mouse MSCs enriched from bone marrow via a method we developed based on immunodepletion. In the latter case, bone marrow cells were expanded in culture for 7~10 days and then subjected to three rounds of immunodepletion using antibodies against CD11b, CD34, and CD45 (20). This approach yields a population of primary cells with a normal karyotype that are devoid of contaminating hematopoietic cells, expresses markers typical of MSCs including CD29, CD44, CD106, and Sca1, exhibit the capacity for tri-lineage differentiation in vitro, and bone formation in vivo. Consequently, enrichment of MSCs from mouse bone marrow via immunodepletion dif-

Table 1. Composition of SAGE databases generated from different MSC populations

SAGE library	Tags sequenced	Unique tags ^a	Unknown tags (%) ^b
CFU-F35 ^c	17,767	6,987	1,136 (6.4)
89R ^d	69,937	17,894	5,640 (8.1)
IDmMSCs ^e	59,007	15,815	1,502 (2.5)

^aNumber of SAGE tags that mapped to a unique mRNA sequence.

^bNumber of SAGE tags that failed to match any reliable mRNA sequence.

^cSingle cell-derived colony of human MSCs from donor #35.

^dNon-clonal population of human MSCs derived from donor #35.

^ePrimary mouse MSCs enriched from marrow via immunodepletion.

fers significantly from many other reported techniques, which typically rely on long-term culture expansion of plastic adherent cells to remove contaminating hematopoietic lineages and results in the generation of immortalized cell lines (21).

A detailed description of the aforementioned SAGE databases has been published previously (22-24). Analysis of these databases revealed that the MSC transcriptome reflected both the developmental potential of MSCs as depicted by the mesengenic process and a capacity to regulate a broad array of biological processes important for bone and marrow function (Fig. 1). In the former case a large number of transcripts were catalogued that play an important role in the specification of mesoderm during development and are also characteristically expressed by skeletal, adipose, and muscle tissue consistent with the capacity of cells to differentiate into various connective tissue cell types. In the latter case, MSCs were also found to express different classes of regulatory proteins important for angiogenesis, cell motility and communication, hematopoiesis, neural activities and immunity and defense. In the process of validating the SAGE data, it became evident that expression of different regulatory proteins was restricted to specific subpopulations of MSCs (24). Accordingly, we hypothesized that these subpopulations exhibit unique biological characteristics and as such contribute to the

broad therapeutic efficacy of MSCs at the population level. Experimental findings recently published from my laboratory, summarized briefly below, support this hypothesis.

Engraftment of MSCs in the central nervous system

Previously, my laboratory reported that immunodepleted MSCs injected intracranially into newborn mice durably engrafted and disseminated throughout a large volume of the brain, and that a small number of engrafted MSCs adopted characteristics of neural cells (25). Various groups have confirmed and extended these findings by demonstrating that MSCs exhibit some measurable degree of therapeutic efficacy in various animal models of neurologic disease (26). These findings have fostered the use of MSCs in clinical trials to treat stroke (27). Regardless, few if any studies have examined in detail the molecular mechanisms that govern MSC engraftment, survival, and migration within the CNS despite the fact that cells typically exhibit poor overall engraftment levels when transplanted to brain tissue.

To explore this question in more detail, we quantified engraftment levels and analyzed the anatomical distribution of MSCs injected intracranially into newborn vs. adult mice over an extended time course (25, 28, 29). In

Fig. 1. The MSC transcriptome. Schematic summarizes the biological nature and/or function of transcripts catalogued via SAGE analysis of human and mouse MSCs. The transcripts include those that regulate mesoderm specification during development or that are characteristically expressed by connective tissues, such as bone, cartilage, adipose, and muscle and as such reflect the developmental potential of MSCs. Also identified were transcripts that regulate various biological processes necessary for bone and marrow function including angiogenesis, cell motility and communication, hematopoiesis, neural activities, immunity and defense. Reprinted with permission from *Stem Cells* (25).

these studies brain tissue harvested at various times post-injection was sliced into coronal sections and used to prepare genomic DNA, which was then analyzed via real-time PCR using a probe that hybridized to sequences in the mouse Y chromosome (28). This approach allowed the amount and distribution of male DNA to be quantified throughout the brains of female mice injected with male MSCs. These studies revealed several key facts about MSC engraftment in the brain. First, regardless of the age of the transplant recipient, only a small fraction of the total number of MSCs injected were detectable in brain several days post-transplant. Therefore, overall survival of MSCs in brain was poor even though injections were performed at relatively low cell densities ($\sim 20,000$ cells/ μ l). Second, significantly higher engraftment levels were observed at later time points in newborn vs. adult transplant recipients, which resulted from the preferential expansion of MSCs engrafted in the brains of newborn mice. This expansion, however, occurred predominantly during the first few months of post-natal life and appeared to parallel normal brain growth and development since at later time points (60–150 days post-transplant) male DNA levels did not vary significantly between transplant recipients. This result was consistent with the complete absence of tumor formation, which in some cases was monitored up to 1 year post-transplant. Third, the anatomical distribution of engrafted MSCs in brain appeared non-random. This conclusion was based on the finding that the amount of male DNA, which was widely distributed along the brain neuraxis of both neonatal and adult transplant recipients, varied significantly between coronal brain slices of individual transplant recipients. Subsequent analysis of tissue sections using a fluorescent mouse Y chromosome paint probe confirmed that engrafted male cells localized to specific anatomical structures such as the striatum, cerebral cortex, and granular layers of the hippocampus and cerebellum (29).

These experiments were repeated in Rhesus macaques as part of a pre-clinical study to evaluate the safety of direct intracranial transplantation of MSCs for a therapeutic intent (30, 31). Herein, unmatched MSCs from a universal male donor were injected intracranially into the caudate putamen of female recipients and at various times post-transplant effects on neural development, behavior, motor performance, and cognition were evaluated. As noted above, after sacrifice the brain tissue of each transplant recipient was cut into coronal slices, which were further subdivided into specimens of comparable size and anatomical location. Genomic DNA from these specimens was then analyzed using a real-time PCR assay that targets the

Macaca sp. Y chromosome (30), and this data was transposed onto a physical map of the macaque brain to visualize the anatomical distribution of engrafted MSCs (31). Similar to that seen in mice, MSC engraftment levels were on average 17.8-fold higher in infant vs. young adult macaque recipients with a maximal observed difference of 180-fold. Moreover, male DNA was found to be widely distributed along the brain neuraxis, and its precise anatomical location overlapped significantly between transplant recipients in both age groups. Infant macaques exhibited the highest engraftment levels in tissue specimens encompassing the somato-sensory, primary motor, and auditory cortex, caudate putamen, striatum, and hippocampus. Moreover, engraftment levels averaged over all transplant recipients varied significantly between different anatomical locations in brain and as a function of time post-injection, indicating that cell engraftment was non-random (Fig. 2) (31). A battery of age- and species-appropriate tests failed to reveal any adverse effects of MSC transplantation on the general health of animals or their cognitive development, motor function or behavior even though the tests were performed during the first year of life, a period of rapid development. Therefore, the procedure was well tolerated in both infant and young adult macaques.

MSC sub populations express neural adhesion and receptor proteins that regulate interneuron migration in brain.

Similarities in the engraftment levels, kinetics, and anatomical distribution of MSCs injected into the brains of mice and Rhesus macaques suggested that a conserved mechanism regulated these processes. It is well established that adherent cells including MSCs must bind to specific extracellular matrix proteins via receptor mediated processes to ensure survival. Attachment to a given substrate invokes the formation of stress fibers that activate cytoskeletal signaling complexes to repress apoptosis (32). Although MSCs express receptors for an array of extracellular matrix proteins common in connective tissues including fibronectin, osteopontin, and collagens these proteins are expressed at low levels in brain, which may account for the poor survival of MSCs following direct intracranial transplantation.

To determine if MSCs express specific adhesion or receptor proteins that may facilitate engraftment in brain tissue, we again interrogated their transcriptome. This analyses revealed that MSCs expressed mRNAs encoding the neural adhesion molecules *ninjurin 1* and *cadherin 2*

Fig. 2. MSCs injected intracranially into infant macaques. (A) Schematic showing the average number of male MSCs, which ranged between 1 and 2,500 cells (colored bar), contained within equivalent brain specimens harvested from a total of eight transplant recipients. MSCs were injected into the caudate nucleus (arrow) of infant macaques at between 6~8 weeks of age and brain tissue was harvested at 3 and 6 months post-transplant. Brain specimens with overlapping engraftment between 2 (diagonal lines bordered in black) or more than 3 (hatched lines bordered in red) transplant recipients are denoted. Regions in white contained no detectable male DNA. The arrow points to the approximate region where MSCs were injected into the brain. (B) Plotted is the average (mean $\pm$ SD) number of MSCs contained within different brain specimens from the same coronal slice of all eight transplant recipients. Significant differences in overall engraftment levels were evident between brain specimens containing between 1~50 (blue), 51~250 (neon) or 251~2,500 (Green, Yellow, Red) MSCs, *, $p < 0.05$. (C) Plotted is the average (mean $\pm$ SD) male DNA levels contained within each respective 3 mm coronal brain from all eight transplant recipients, *, $p < 0.05$, **, $p < 0.01$. (D) Plotted is the average (mean $\pm$ SD) male DNA levels contained within each respective 3 mm coronal brain slice from all eight transplant recipients sacrificed at 3 or 6 months post-injection, *, $p < 0.05$, **, $p < 0.001$. Coronal brain slices are numbered 1~13 in a rostral- to-caudal orientation. Reprinted with permission from *Stem Cells* (31).

(CDH2) and the guidance receptor proteins neogenin 1 (NEO1), neuropilin 1 (NRP1), neuropilin 2 (NRP2), and roundabout homologs 1 (ROBO1) and 4 (ROBO4) (31). Several of these proteins have been previously shown to be expressed by marrow cells. For example, CDH2 plays an important role in modulating cell-to-cell adhesion and cellular differentiation of osteoblasts (33) and ROBO1 was reported to regulate leukocyte chemotaxis in response to secreted Slit proteins (34). We demonstrated that expression of the aforementioned proteins was conserved in mouse, non-human primate, and human MSCs, suggesting an important role of these proteins in bone and marrow (29, 31). Moreover, expression of these proteins was shown to be restricted to specific MSC subpopulations, which may impart cells with a unique capacity to engraft in brain and migrate in response to expressed guidance cues.

In support of this hypothesis, we demonstrated that binding of MSCs to plates coated with CDH2 or netrin 1 was dose dependent and could be specifically inhibited by soluble CDH2 or a neutralizing antibody against NEO1 (the receptor for netrin 1), respectively. Therefore, CDH2 and netrin 1 function as homo- and heterophilic adhesion molecules, respectively, in MSCs. MSC migration was also shown to be stimulated by semaphorin 3A or repulsive guidance molecule A, which are ligands for the NRP1 and NEO1 receptors, respectively, and inhibited by neutralizing antibodies to these receptors (31). Most recently we isolated a sub population of CDH2^{+ve} mouse MSCs and showed that following intracranial injection, these cells exhibit 3-fold higher engraftment levels as compared to CDH2^{-ve} cells [DG Phinney] unpublished data, 2009). CDH2^{+ve} vs. CDH2^{-ve} cells also exhibited a non-overlapping distribution in the brain. These data are consistent with CDH2 playing a role in regulating MSC engraftment and/or migration within the CNS. Accordingly, the aforementioned subpopulations may represent novel cellular vectors that exhibit enhanced engraftment in brain and can be targeted to specific anatomical brain regions for a therapeutic intent.

MSC subpopulations possess anti-inflammatory activity

My laboratory in collaboration with researchers at the University of Pittsburgh was one of the first to report that systemic administration of MSCs ameliorated bleomycin-induced lung injury in mice (35). Initially, the mechanism for this affect was unclear, but appeared to be related to the timing of MSC administration following bleomycin challenge. Specifically, we found that the extent of in-

flammation and fibrosis in lung tissue was significantly reduced in mice that were administered MSCs at the time of bleomycin challenge as compared to those exposed to bleomycin alone. In stark contrast, the degree of inflammation and extent of fibrosis was unaltered if animals were administered MSCs seven days after bleomycin challenge. Therefore, MSCs exerted a prominent therapeutic effect during the early phase of lung injury when the inflammatory response is at a peak. Consequently, we interrogated the MSC transcriptome to determine if cells expressed proteins with anti-inflammatory activity. This analysis identified interleukin 1 receptor antagonist (IL1RN) as a potential candidate. The mouse transcriptome contained several SAGE tags corresponding to IL1RN, one of which ranked in the top 100 of all catalogued tags (36). Screening of a mouse MSC cDNA library confirmed that cells expressed transcripts encoding IL1RN, and ELISA analysis of conditioned media confirmed they secreted appreciable amounts of IL1RN protein. In addition, FACS analysis of whole bone marrow cells revealed that MSCs were the principle source of IL1RN in bone marrow, but that the protein was expressed by only 24% of the population. Similarly, we showed that approximately 5% of human MSCs also expressed IL1RN (36).

Subsequently, we demonstrated that MSC conditioned media containing known amounts of IL1RN was equally effective as purified, recombinant IL1RN protein in inhibiting the growth of an IL-1 dependent T lymphocyte cell line (D10.G4.1) in vitro. Moreover, this inhibitory effect of conditioned media could be completely abrogated by addition of a neutralizing anti-IL1RN antibody (36). Subsequently, we demonstrated that MSC administration abrogated the inflammatory response in vivo induced in lung tissue by exposure to bleomycin. Specifically, we showed that at 3 days post-exposure to bleomycin, bronchoalveolar lavage (BAL) fluid collected from animals administered bleomycin and MSCs contained significantly fewer neutrophils and lower levels of IL1RN protein as compared to animals challenged with bleomycin alone. Similarly, MSC administration inhibited expression of TNF- α , IL-1 α , and IL1RN mRNA expression in lung tissue 7 and 14 days post-exposure, each of which were dramatically up regulated at these time points in response to bleomycin exposure.

Based on these data we proposed the following mechanism to explain, in part, the therapeutic effect of MSCs in a mouse model of pulmonary fibrosis (Fig. 3). Bleomycin exposure in mice is known to activate macrophages resident in lung tissue to secrete TNF- α and IL-1, which induce a pro-inflammatory signaling cascade resulting in

Fig. 3. IL1RN expression by MSCs ameliorates the inflammatory response in injured lung. Bleomycin induced lung injury promotes macrophages resident in lung tissue to secrete IL-1 α and TNF- α , thereby promoting a pro-inflammatory response (red arrows). Cytokines produced by activated macrophages recruit other immune cells into lung tissue, which also secrete inflammatory cytokines and proteases that alter the lung cyto-architecture and induced apoptosis or resident epithelial cells (red crosses). The pro-inflammatory response induced by bleomycin is counterbalanced by the anti-inflammatory effects (blue arrows) of IL1RN produced by engrafted MSCs. Production of IL1RN by MSCs may be induced by IL-1 α , which may further enhance their anti-inflammatory effects. Reprinted by permission from *Proc Natl Acad Sci USA* (35).

recruitment of other immune cells into lung from the circulation. The infiltrating neutrophils and lymphocytes release other pro-inflammatory cytokines and proteases that augment the inflammatory response, alter the lung cyto-architecture, and induce apoptosis of resident alveolar epithelial cells. Based on our experimental data, we hypothesized that MSCs engrafted in lung secrete appreciable levels of IL1RN, and possibly other unidentified anti-inflammatory factors, which effectively short circuit the inflammatory response by antagonizing the activity of IL-1. This potent anti-inflammatory effect of MSCs is likely enhanced by the fact that the cells engraft within the lungs for prolonged periods after systemic injection (37, 38) and engraftment levels are further elevated in lung by BLM exposure (35). Our analysis also revealed that exposure to IL-1 α up regulates expression of IL1RN in mouse MSCs (36). This finding is consistent with other studies showing that IL-1 α positively regulates expression of IL1RN to counterbalance its potent activity in vivo (39). As such, IL1RN expression levels within the injured lung should be proportional to the amount of IL-1 α activity

induced in response to bleomycin exposure. This fact is consistent with our in vivo data, which revealed that animals administered MSCs at the time of bleomycin exposure maintained low levels of IL1RN expression in lung, providing further evidence that engrafted MSCs suppressed the inflammatory response. Recently, we have compared the cytokine/chemokine expression profile of IL1RN⁺ vs. IL1RN⁻ human MSC subpopulations enriched by fluorescence activated cell sorting. This analysis revealed that IL1RN⁺ cells express chemokines that regulate chemotaxis of various immune cell types, whereas the IL1RN⁻ population expressed a large number of proteins related to the IL-1 family ([DG Phinney], unpublished data, 2009). Further characterization of these subpopulations should reveal further insight into how MSCs interact with the immune system and regulate inflammation induced by tissue injury.

Stromal vs. stem cell

A rapidly growing body of literature has extolled the

therapeutic effects of MSCs in various animal models of disease and in human clinical trials. Initially, the potency of MSCs in these applications was attributed to their stem cell-like characteristics. However, more recent data suggest that their therapeutic effect results from paracrine signaling, which alters the tissue microenvironment in a manner conducive to healing and regeneration. Our SAGE analysis of the MSC transcriptome provides mechanistic insight into these effects by demonstrating that MSC populations express a diverse array of regulatory proteins important for bone and marrow function but that may serendipitously promote tissue repair and regeneration following transplantation of MSCs to ectopic sites *in vivo*. For example, MSCs express a variety of neurotrophins, neurite inducing factors, and other neuro-regulatory proteins that likely participate in the maintenance of nervous tissue and guide the innervation of nerve fibers in bone and marrow during growth, remodeling, and reparation after injury. Some neuro-regulatory proteins are also known to effect the growth and differentiation of hematopoietic cells, as well. Expression of these factors likely contributes to the capacity of MSCs to promote repair of nervous tissue following spinal cord injury, stroke, or traumatic brain injury (26). Similarly, we have shown that MSCs express a variety of angiogenic factors and other proteins that regulate endothelial cell growth and motility (40). Co-operatively, these factors induce capillary proliferation and expansion of the sinusoidal space, processes essential for bone growth. They likely also contribute significantly to the therapeutic effect of MSCs following myocardial infarction. Additionally, IL-1 α is known to be a potent mediator of bone turnover, providing a rationale for stromal subtypes that express IL1RN to modulate its activity *in vivo*. In the appropriate context, we have shown that IL1RN-expressing MSCs may have potent therapeutic effects by virtue of their ability to antagonize the activity of IL-1 α and block the inflammatory response that ensues following tissue injury (35, 36). Despite the fact that MSC populations uniformly express various surface antigens, our data clearly demonstrate that populations are functionally heterogeneous and that this heterogeneity contributes to their broad therapeutic activity *in vivo*. Further analysis of the unique biology of these subpopulations will offer new insights into the complexity of marrow stroma and its varied functions, but may also generate subpopulations that function as more potent cellular vectors to treat disease.

Acknowledgements

The author would like to acknowledge Dr. Luis A.

Ortiz, Dr. Iryna A. Isakova, Maria Dutreil, and Melody Baddoo for their important contributions to the work detailed in this review. This research was supported in part by grants from the National Institutes of Health (DGP, R01 NS052301 and R01 NS039033; LAO HL073771), the Louisiana Board of Reagents Millennium Health Education Fund (DGP), and the Louisiana Gene Therapy Research Consortium.

Potential Conflict of Interests

The author has not potential conflicts of interest to report.

References

1. Phinney DG. Building a consensus regarding the nature and origin of mesenchymal stem cells. *J Cell Biochem Suppl* 2002;38:7-12
2. Dexter TM, Allen TD, Lajtha LG. Conditions controlling the proliferation of haemopoietic stem cells *in vitro*. *J Cell Physiol* 1977;91:335-344
3. Gartner S, Kaplan H. Long-term culture of human bone marrow cells. *Proc Natl Acad Sci U S A* 1980;77:4756-4759
4. Phinney DG. Marrow stem cells. In: Polak J, editor. *Advances in Tissue Engineering*. London: Imperial College Press; 2008. 95-122.
5. Beresford JN, Bennet JH, Devlin C, Leboy PS, Owen ME. Evidence for an inverse relationship between the differentiation of adipocytic and osteogenic cells in rat marrow stromal cell cultures. *J Cell Sci* 1992;102:341-351
6. Umezawa A, Maruyama T, Segawa K, Shadduck RK, Waheed A, Hata JI. Multipotent marrow stromal cell line is able to induce hematopoiesis *in vivo*. *J Cell Physiol* 1992;151:197-205
7. Caplan AI. The mesengenic process. *Clin Plast Surg* 1994;21:429-435
8. Dennis JE, Merriam A, Awadallah A, Yoo JU, Johnstone B, Caplan AI. A quadripotential mesenchymal progenitor cell isolated from the marrow of an adult mouse. *J Bone Miner Res* 1999;14:700-709
9. Pittenger MF, MacKay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. *Science* 1999;284:143-147
10. Digirolamo CM, Stokes D, Colter D, Phinney DG, Class R, Prockop DJ. Propagation and senescence of human marrow stromal cells in culture: a simple colony-forming assay identifies samples with the greatest potential to propagate and differentiate. *Br J Haematol* 1999;107:275-281
11. Mauraglia A, Cancedda R, Quarto R. Clonal mesenchymal progenitors from human bone marrow differentiate *in vitro* according to a hierarchical model. *J Cell Sci* 2000;113:1161-1166
12. Kuznetsov SA, Krebsbach PH, Satomura K, Kerr J, Riminucci M, Benayahu D, Robey PG. Single-colony derived

- strains of human marrow stromal fibroblasts form bone after transplantation in vivo. *J Bone Miner Res* 1997;12:1335-1347
13. Miller-Sieburg C, Deryugina E. The stromal cells' guide to the stem cell universe. *Stem Cells* 1985;13:477-486
  14. Pittenger MF, Mbalaviele G, Black M, Mosca JD, Marshak DR. 2001. Mesenchymal stem cells. In: Koller MR, Palsson BO, Master JRW, editors. *Human Cell Culture*. Great Britain: Kluwer Academic Publishers; 189-207
  15. Quirici N, Soligo D, Bossolasco P, Servida F, Lumini C, Delilieri GL. Isolation of bone marrow mesenchymal stem cells by anti-nerve growth factor receptor antibodies. *Expt Hematol* 2002;30:783-791
  16. Buhring HJ, Battula VL, Trem S, Schewe B, Kanz L, Vogel W. Novel markers for the prospective isolation of human MSC. *Ann N Y Acad Sci* 2007;1106:262-271
  17. Gang EJ, Bosnakovski D, Figueiredo CA, Visser JW, Perlangeiro RC. SSEA-4 identified mesenchymal stem cells from bone marrow. *Blood* 2007;109:1743-1751
  18. Sacchetti B, Funari A, Michienzi S, Di Cesare S, Piersanti S, Saggio I, Tagliafico E, Ferrari S, Robey PG, Riminucci M, Bianco P. Self-renewing osteoprogenitors in bone marrow sinusoids can organize a hematopoietic microenvironment. *Cell* 2007;131:324-336
  19. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop DJ, Horwitz E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 2006;8:315-317
  20. Baddoo M, Hill K, Wilkinson R, Gaupp D, Hughes C, Kopen GC, Phinney DG. Characterization of mesenchymal stem cells isolated from murine bone marrow by negative selection. *J Cell Biochem* 2003;89:1235-1249
  21. Phinney DG. 2008. Isolation of murine mesenchymal stem cells by immunodepletion. In: Prockop DJ, Bunnell BA, Phinney DG, editors. *Methods in Molecular Biology, Molecular Medicine and Biotechnology: Adult Mesenchymal Stem Cells*. Totowa NJ: Humana Press Inc; 171-186
  22. Tremain N, Korkko J, Ibberson D, Kopen GC, DiGirolamo C, Phinney DG. MicroSAGE analysis of 2,353 expressed genes in a single cell-derived colony of undifferentiated human mesenchymal stem cells reveals mRNAs of multiple cell lineages. *Stem Cells* 2001;19:408-418
  23. Phinney DG. 2006. Gene expression profiles of mesenchymal stem cells. In: Nolte JA, editor. *Genetic Engineering of Mesenchymal Stem Cells*. The Netherlands: Springer; 59-80
  24. Phinney DG, Hill K, Michelson C, DuTreil M, Hughes C, Humphries S, Wilkinson R, Baddoo M, Bayly E. Biological activities encoded by the murine mesenchymal stem cell transcriptome provide a basis for their developmental potential and broad therapeutic efficacy. *Stem Cells* 2006; 4:186-198
  25. Kopen G, Prockop DJ, Phinney DG. Marrow stromal cells migrate throughout forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brains. *Proc Natl Acad Sci U S A* 1999;96:10711-10716
  26. Phinney DG, Isakova I. Plasticity and therapeutic potential of mesenchymal stem cells in the nervous system. *Curr Pharm Design* 2005;11:1255-1265
  27. Jeun SS. Clinical application of mesenchymal stem cells for CNS disease. 5th Catholic International Stem Cell Symposium Proceedings, Seoul, Korea 2007; pp48-49
  28. McBride C, Gaupp D, Phinney DG. Quantifying levels of transplanted murine and human mesenchymal stem cells in vivo by real-time PCR. *Cytotherapy* 2003;5:7-18
  29. Phinney DG, Baddoo M, Gaupp D, DuTreil M, Isakova IA. Murine MSCs transplanted to the CNS of neonatal vs. adult mice exhibit distinct engraftment kinetics and express receptors that guide neuronal cell migration. *Stem Cell Dev* 2006;15:437-447
  30. Isakova IA, Baker K, Dufour J, Gaupp D, Phinney DG. Preclinical evaluation of adult stem cell engraftment and toxicity in the CNS of rhesus macaques. *Mol Therapy* 2006;13:1173-1184
  31. Isakova IA, Baker K, Dufour J, Gaupp D, Phinney DG. Age- and dose related effects on MSC engraftment levels and anatomical distribution in the CNS of nonhuman primates: identification of novel MSC subpopulations that respond to guidance cues in brain. *Stem Cells* 2007;25:3261-3270
  32. Matter ML, Ruoslahti E. A signaling pathway from the alpha5beta1 and alpha(v)beta3 integrins that elevates bcl-2 transcription. *J Biol Chem* 2001;276:27757-27763
  33. Cheng SL, Shin CS, Towler DA, Civitelli R. A dominant negative cadherin inhibits osteoblast differentiation. *J Bone Miner Res* 2000;15:2362-2370
  34. Wu JY, Feng L, Park HT, Havlioglu N, Wen L, Tang H, Bacon KB, Jiang Zh, Zhang Xc, Rao Y. The neuronal repellent Slit inhibits leukocyte chemotaxis induced by chemotactic factors. *Nature* 2001;410:948-952
  35. Ortiz LA, Gambelli F, McBride C, Gaupp D, Baddoo M, Kaminski N, Phinney DG. Mesenchymal stem cell engraftment in lung is enhanced in response to bleomycin exposure and ameliorates its fibrotic effects. *Proc Natl Acad Sci U S A* 2003;100:8407-8411
  36. Ortiz LA, DuTreil M, Fattman C, Pandey AC, Torres G, Go K, Phinney DG. Interleukin 1 receptor antagonist mediates the anti-inflammatory and anti-fibrotic effect of mesenchymal stem cells during lung injury. *Proc Natl Acad Sci U S A* 2007;104:11002-11007
  37. Rochefort GY, Vaudin P, Bonnet N, Pages JC, Domenech J, Charbord P, Eder V. Influence of hypoxia on the domiciliation of mesenchymal stem cells after infusion into rats: possibilities of targeting pulmonary artery remodeling via cells therapies? *Respir Res* 2005;6:125
  38. Fischer UM, Harting MT, Jimenez F, Monzon-Posadas WO, Xue H, Savitz SI, Laine GA, Cox CS. Pulmonary passage is a major obstacle for intravenous stem cell delivery: The pulmonary first pass effect. *Stem Cells Dev* 2008[Epub ahead of print]
  39. Juge-Aubry CE, Somm E, Chicheportiche R, Burger D, Perin A, Cuénod-Pittet B, Quinodoz P, Giusti V, Dayer JM,

Meier CA. Regulatory effects of interleukin (IL)-1, interferon-beta, and IL-4 on the production of IL-1 receptor antagonist by human adipose tissue. *J Clin Endocrin Metab* 2004;89:2652-2658

40. Phinney DG. Biochemical heterogeneity of mesenchymal stem cell populations: clues to their therapeutic efficacy. *Cell Cycle* 2007;6:2884-2889