Mesenchymal Progenitor Cell Research

Limitations and Recommendations

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There is considerable interest in mesenchymal stem cell biology. This relates, in part, to the possibility that such cells could be used to replace tissues in degenerative diseases of mesenchyme. In this article, the current status of the field is discussed, highlighting technical and theoretical issues related to the identification, isolation, and characterization of these cells. Our focus is the endogenous non-circulating mesenchymal stem cell populations that normally reside in adult organs.

Keywords: mesenchyme; stem cells; progenitor; lung

Mature mesenchyme is composed of a loosely packed collection of cells suspended in a gelatinous network of extracellular matrix. Mesenchymal tissue can be found in all organs and acts to provide structural support, and to regulate trafficking of cells through tissues. Classical mesenchymal cells are fibroblasts, myofibroblasts, and vascular wall cell types, and, as the name implies, these cells principally arise from the middle or mesoderm layer of the embryonic trilaminar germ disc (1). One notable exception to this is in the head, neck, and brachial arch region where so-called mesenchymal cells arise from the neural crest cells of the embryonic ectoderm (2). Mesenchymal cells remain arguably the least well-characterized cell type in the body. This is a direct consequence of the lack of specific markers that can definitively differentiate the diverse and heterogeneous phenotypes contained within mesenchymal cell populations.

Over three decades ago, Friedenstein and colleagues identified the presence of an adherent fibroblast-like population that emerged after placement of bone marrow preparations on tissue culture plastic (3). In this original study, these cells were found to possess an ability to assume osteogenic-like phenotypes, an observation that served as the foundation for future work in the field. The term “mesenchymal stem cell” (MSC) was originally used by Caplan to describe the bone marrow–derived plastic adherent cell, in view of subsequent studies showing that these cells possessed multipotent differentiation capacity (4). In this regard, an abundant literature has gone on to show that individual marrow-derived MSCs serve as precursors to a variety of mesodermal cell types, including bone, cartilage, fat, and muscle (5). Recent findings show that cells with similar capacity reside in virtually all adult and embryonic tissues (6–11). In accordance with this, “MSC” is no longer synonymous with the bone marrow–derived cell type but rather refers to any cell with multipotent mesenchymal differentiation capacity in culture.

To date, the true functional role of MSCs *in vivo* is not clear. Much of the work in this area is focused on elucidating the function of MSCs within the bone marrow. On the basis of a variety of experimental observations, it has been argued that MSCs are

intimately involved in maintaining the local hematopoietic stem cell niche; this activity is believed to be mediated through provision of structural support, and by serving as a source of hematopoietic growth factors (12). Gene expression profiling of marrow MSCs supports the possibility that these cells could be involved in various biological processes, such as vascular formation, bone and cartilage homeostasis, wound repair, and host defense (13, 14). Although it is reasonable to speculate that MSCs from all tissues perform similar activities, the inherent difficulties associated with isolating live cells from solid organs has greatly limited the study of non-hematopoietic-derived MSC populations.

CURRENT ISSUES IN THE FIELD OF MSC RESEARCH

There is a general consensus that the field of MSC biology is significantly less developed than other stem cell fields. This is most notable when comparisons are made to the field of hematopoietic stem cell (HSC) research. It is important to underscore the fact that the impressive advances in HSC research are largely attributable to two factors: (1) the ready availability of a powerful and relevant *in vivo* model and assay system and (2) the wide array of well-developed and functionally relevant markers and isolation methodologies. A brief discussion of these factors will help highlight, at least in part, why MSC-related research has lagged, and what types of tools are needed to advance the field.

Well-defined bone marrow ablation regimens in mice have provided a powerful model to directly test HSC function *in vivo* (15–19). These models, which can be easily adapted in most laboratories, are not dependent on any special expertise. Stem cell activities can be readily assayed in these models by evaluating the capacity of injected cells to permanently reconstitute specific differentiated hematopoietic cell lineages. Remarkably, after intravenous or intra-peritoneal administration, HSCs home to and take residence in the bone marrow.

The wide commercial availability of directly conjugated monoclonal antibodies that bind to well-characterized surface antigens has further enhanced the power of this model. Although there is not a single marker that characterizes HSCs, a constellation of markers can be used to identify such cells (16). Notably, high-speed cell sorting, in which thousands of cells can be analyzed per second, has helped overcome the considerable technical impediment related to the very low frequency of HSCs in marrow preparations. Thus, it is now possible to evaluate the stem cell function of a single isolated phenotypically distinct marrow cell in marrow-ablated mice (20).

In striking contrast, there is no comparable *in vivo* model to evaluate MSC function. In large part, cells are designated as MSCs if they can assume distinct differentiated mesenchymal cell types (e.g., bone, fat, cartilage) after transfer to defined culture media *in vitro* (5, 6, 21, 22). Whether *in vitro* differentiation capacity is physiologically relevant remains unclear and, at present, is a key issue for the field. Interestingly, we found that serially passaged fibroblast cell lines harbor a relatively high frequency of cells that can assume differentiated phenotypes, if placed in the appropriate differentiation condition *in vitro* (Figure 1). This

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Figure 1. The MLG fibroblastic cell line (ATCC; American Type Culture Collection, Manassas, VA) displays multipotent differentiation capacity *in vitro*. *Left:* After placement in chondrogenic culture conditions, MLG cells form aggregated cellular structures that contain an abundance of proteoglycans as determined by positive Toluidine blue/purple stain. *Middle:* In osteogenic inducing medium, cells exhibit a mineralization capacity as determined by positive Alizarin Red S staining. *Right:* After transfer to adipogenic conditions, Oil Red O–positive fat droplets (red) become visible.

observation necessarily raises questions regarding (1) the basic validity and meaning of the *in vitro* MSC differentiation assay system and (2) what is the most appropriate comparative control cell population for MSC studies. It is noteworthy, therefore, that this latter issue is not addressed or considered in many MSC-related publications (5, 6, 10, 21).

Various cell surface markers for MSCs have been identified (23, 24). Furthermore, a list of cell surface epitopes that collectively are associated with marrow-derived MSCs has been created by the International Society for Cellular Therapy (24). To date, the usefulness of these markers has been somewhat limited because of a lack of specificity. This state of affairs remains a basic challenge for the field to surmount. Similar to HSCs, it is likely that there will not be a single marker that specifies an MSC. It may be, however, that, like HSCs, a collection of markers could be used for identification and isolation purposes (25).

Another major limiting issue for the field is the fact that the study of MSCs requires prolonged culturing of dispersed cell populations on plastic (5, 26–28). As discussed, Friedenstein and colleagues originally performed this on bone marrow aspirates, identifying the classic MSC population (3). Using this or related methodology, MSC-like cells can be isolated from multiple tissue sites (7, 8, 17). The adherent marrow cell population contains at least three distinct cell types: CD45 myeloid cells, endothelial-like cells, and classic MSCs. Adherent cell preparations can be further purified by negative selection (29). Collectively, these observations raise important questions such as what is the effect of prolonged growth on plastic on MSC phenotype, and in turn what is the precise relationship between cells derived in this manner and their *in vivo* counterpart.

Although these issues may limit their usefulness from an experimental and biological perspective, cultured marrow MSCs preparations have shown promise as a cell-based therapy reagent (27, 30–33). Indeed, injected MSCs can serve as precursors to cartilage and bone after direct *in vivo* injection (30, 32, 34, 35). Albeit controversial, it has also been reported that these cells may serve as precursors to lung epithelium, neural tissues, and heart (22, 31, 36).

In mice, a potentially beneficial therapeutic role for MSCs that does not relate to direct tissue replacement has been noted in injury models in heart, skin, lung, and nervous system (22, 31, 33, 36, 37, 38). In these studies, it has been proposed that injected MSCs stimulate endogenous stem cell activity, down-

regulate immune responses, and serve as a source of key soluble trophic factors (22, 39, 40). It is important to again note, however, that these types of observations do not necessarily confirm that MSCs have similar functions during homeostasis or that these activities are unique to undifferentiated mesenchymal cell populations (41).

ADULT LUNG MSCs

The adult lung contains a variety of mesenchymal components (cartilage, smooth muscle, and myofibroblasts). Whether and how these cells renew in the adult lung is poorly understood. It is conceivable that these components are replaced through a mechanism that does not involve a progenitor cell intermediate but rather through the proliferation and subsequent expansion of already differentiated cells. Indeed, there is evidence to suggest that the excess bronchial smooth muscle that accumulates around the asthmatic airway may be due to local proliferation of mature smooth muscle cells (42). As with the MSC field in general, lung mesenchymal progenitor cell biology has been limited by the lack of tools and markers (7, 11, 41). The difficulty in studying the turnover and replacement of the lung's mesenchymal compartments is further limited by the fact that these tissues are intrinsically very quiescent. Using conventional methodology, an MSC-like cell can be derived from the adult lung (7, 41).

Because markers found on MSCs are not informative, we sought to develop an alternative strategy to isolate and characterize adult lung mesenchymal progenitor cells. Accumulated data indicate that stem and progenitor cells in multiple tissues are markedly enriched in populations that have the capacity to efflux vital fluorescent dyes, such as Hoechst (16, 17). Taking advantage of this property, we found that a candidate mesenchymal progenitor could be isolated by flow cytometry from the adult lung. *In vitro* assays confirmed that this cell could give rise to smooth muscle, fat, cartilage, and bone (41). In contrast, noneffluxing lung cells with the same surface phenotype did not display this differentiation capability. Additional work using chimeric mice suggests that this putative lung mesenchymal progenitor cell is not derived from the circulating blood, as has been postulated for other mesenchymal progenitors (41). As with other studies in the field, the *in vivo* biological significance of our findings remains uncertain.

Notably, a side-by-side comparison of lung and classical marrow MSCs has not yet been performed. On the basis of pub-

lished literature, these cells share some similar surface epitopes (e.g., Sca-1, CD106), and both lack expression of mature hematopoietic markers (41, 43). Our data suggest that marrow MSCs, however, do not efflux vital dyes (41).

The precise localization of lung mesenchymal progenitor cells remains to be determined. Of interest, there are data suggesting that MSC-like cells localize to perivascular sites (44). In fat, MSCs are believed to reside in a subendothelial site, whereas in the bone marrow, they are found in sinusoids where they contribute to the hematopoietic stem cell niche (45). Indeed, transplantation of osteoprogenitors derived from marrow sinusoids can establish functional hematopoietic niches in non-marrow locations (44).

CONCLUSIONS

The state of MSC research has been significantly hampered by a lack of basic tools. Overall, the types of reagents and methods that helped advance the field of HSC research can guide, at least in part, what is needed for the MSC field. New reagents and tools would also necessarily provide a means to address key unanswered questions, such as where are MSCs localized, do they circulate, what is their frequency, what is the relation between MSCs derived from different tissue sites, and do MSCs serve other unexpected functions *in vivo*? Finally, it is incumbent on the field to fundamentally address how best to define an MSC, because the current definition fundamentally rests, in part, on an *in vitro* assay system that is fraught with issues of specificity and *in vivo* relevance.

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References

- Nichols DH. Mesenchyme formation from the trigeminal placodes of the mouse embryo. *Am J Anat* 1986;176:19–31.
- Nichols DH. Formation and distribution of neural crest mesenchyme to the first pharyngeal arch region of the mouse embryo. *Am J Anat* 1986;176:221–231.
- Friedenstein AJ, Gorskaja JF, Kulagina NN. Fibroblast precursors in normal and irradiated mouse hematopoietic organs. *Exp Hematol* 1976;4:267–274.
- Caplan AI. The mesengenic process. *Clin Plast Surg* 1994;21:429–435.
- Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multi-lineage potential of adult human mesenchymal stem cells. *Science* 1999;284:143–147.
- Mendes SC, Robin C, Dzierzak E. Mesenchymal progenitor cells localize within hematopoietic sites throughout ontogeny. *Development* 2005;132:1127–1136.
- Fan CG, Tang FW, Zhang QJ, Lu SH, Liu HY, Zhao ZM, Liu B, Han ZB, Han ZC. Characterization and neural differentiation of fetal lung mesenchymal stem cells. *Cell Transplant* 2005;14:311–321.
- Sabatini F, Petecchia L, Taviani M, Jodon de Villeroche V, Rossi GA, Brouty-Boye D. Human bronchial fibroblasts exhibit a mesenchymal stem cell phenotype and multilineage differentiating potentialities. *Lab Invest* 2005;85:962–971.
- Toma JG, Akhavan M, Fernandes KJ, Barnabe-Heider F, Sadikot A, Kaplan DR, Miller FD. Isolation of multipotent adult stem cells from the dermis of mammalian skin. *Nat Cell Biol* 2001;3:778–784.
- Young HE, Steele TA, Bray RA, Hudson J, Floyd JA, Hawkins K, Thomas K, Austin T, Edwards C, Cuzzourt J, et al. Human reserve pluripotent mesenchymal stem cells are present in the connective tissues of skeletal muscle and dermis derived from fetal, adult, and geriatric donors. *Anat Rec* 2001;264:51–62.
- Lama VN, Smith L, Badri L, Flint A, Andrei AC, Murray S, Wang Z, Liao H, Toews GB, Krebsbach PH, et al. Evidence for tissue-resident mesenchymal stem cells in human adult lung from studies of transplanted allografts. *J Clin Invest* 2007;117:989–996.
- Yin T, Li L. The stem cell niches in bone. *J Clin Invest* 2006;116:1195–1201.
- 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;24:186–198.
- 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.
- Spangrude GJ, Heimfeld S, Weissman IL. Purification and characterization of mouse hematopoietic stem cells. *Science* 1988;241:58–62.
- Goodell MA, Rosenzweig M, Kim H, Marks DF, DeMaria M, Paradis G, Grupp SA, Sieff CA, Mulligan RC, Johnson RP. Dye efflux studies suggest that hematopoietic stem cells expressing low or undetectable levels of CD34 antigen exist in multiple species. *Nat Med* 1997;3:1337–1345.
- Summer R, Kotton DN, Liang S, Fitzsimmons K, Sun X, Fine A. Embryonic lung side population cells are hematopoietic and vascular precursors. *Am J Respir Cell Mol Biol* 2005;33:32–40.
- Kotton DN, Fabian AJ, Mulligan RC. A novel stem-cell population in adult liver with potent hematopoietic-reconstitution activity. *Blood* 2005;106:1574–1580.
- Morrison SJ, Uchida N, Weissman IL. The biology of hematopoietic stem cells. *Annu Rev Cell Dev Biol* 1995;11:35–71.
- Camargo FD, Green R, Capetanaki Y, Jackson KA, Goodell MA. Single hematopoietic stem cells generate skeletal muscle through myeloid intermediates. *Nat Med* 2003;9:1520–1527.
- Caplan AI. Mesenchymal stem cells. *J Orthop Res* 1991;9:641–650.
- Phinney DG, Prockop DJ. Concise review: mesenchymal stem/multipotent stromal cells: the state of transdifferentiation and modes of tissue repair—current views. *Stem Cells* 2007;25:2896–2902.
- Delorme B, Ringe J, Gallay N, Levern Y, Kerboeuf D, Jorgensen C, Rosset P, Sensebe L, Layrolle P, Haupl T, et al. Specific plasma membrane protein phenotype of culture-amplified and native human bone marrow mesenchymal stem cells. *Blood* 2008;111:2631–2635.
- Dominici M, Le BK, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop D, Horvitz E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 2006;8:315–317.
- Chamberlain G, Fox J, Ashton B, Middleton J. Concise review: mesenchymal stem cells: their phenotype, differentiation capacity, immunological features, and potential for homing. *Stem Cells* 2007;25:2739–2749.
- Phinney DG, Kopen G, Isaacson RL, Prockop DJ. Plastic adherent stromal cells from the bone marrow of commonly used strains of inbred mice: variations in yield, growth, and differentiation. *J Cell Biochem* 1999;72:570–585.
- Prockop DJ. Marrow stromal cells as stem cells for nonhematopoietic tissues. *Science* 1997;276:71–74.
- Prockop DJ, Sekiya I, Colter DC. Isolation and characterization of rapidly self-renewing stem cells from cultures of human marrow stromal cells. *Cytotherapy* 2001;3:393–396.
- 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.
- Horwitz EM, Prockop DJ, Fitzpatrick LA, Koo WW, Gordon PL, Neel M, Sussman M, Orchard P, Marx JC, Pyritz RE, et al. Transplantation and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. *Nat Med* 1999;5:309–313.
- Kotton DN, Ma BY, Cardoso WV, Sanderson EA, Summer RS, Williams MC, Fine A. Bone marrow-derived cells as progenitors of lung alveolar epithelium. *Development* 2001;128:5181–5188.
- Pereira RF, O'Hara MD, Laptev AV, Halford KW, Pollard MD, Class R, Simon D, Livezey K, Prockop DJ. Marrow stromal cells as a source of progenitor cells for nonhematopoietic tissues in transgenic mice with a phenotype of osteogenesis imperfecta. *Proc Natl Acad Sci USA* 1998;95:1142–1147.
- Rojas M, Xu J, Woods CR, Mora AL, Spears W, Roman J, Brigham KL. Bone marrow-derived mesenchymal stem cells in repair of the injured lung. *Am J Respir Cell Mol Biol* 2005;33:145–152.
- Horwitz EM, Prockop DJ, Gordon PL, Koo WW, Fitzpatrick LA, Neel MD, McCarville ME, Orchard PJ, Pyritz RE, Brenner MK. Clinical responses to bone marrow transplantation in children with severe osteogenesis imperfecta. *Blood* 2001;97:1227–1231.

35. Pereira RF, Halford KW, O'Hara MD, Leeper DB, Sokolov BP, Pollard MD, Bagasra O, Prockop DJ. Cultured adherent cells from marrow can serve as long-lasting precursor cells for bone, cartilage, and lung in irradiated mice. *Proc Natl Acad Sci USA* 1995;92:4857-4861.
36. Iso Y, Spees JL, Serrano C, Bakondi B, Pochampally R, Song YH, Sobel BE, Delafontaine P, Prockop DJ. Multipotent human stromal cells improve cardiac function after myocardial infarction in mice without long-term engraftment. *Biochem Biophys Res Commun* 2007;354:700-706.
37. 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 USA* 2003;100:8407-8411.
38. Xu J, Woods CR, Mora AL, Joodi R, Brigham KL, Iyer S, Rojas M. Prevention of endotoxin-induced systemic response by bone marrow-derived mesenchymal stem cells in mice. *Am J Physiol Lung Cell Mol Physiol* 2007;293:L131-L141.
39. Ortiz LA, DuTreil M, Fattman C, Pandey AC, Torres G, Go K, Phinney DG. Interleukin 1 receptor antagonist mediates the antiinflammatory and antifibrotic effect of mesenchymal stem cells during lung injury. *Proc Natl Acad Sci USA* 2007;104:11002-11007.
40. Prockop DJ. "Stemness" does not explain the repair of many tissues by mesenchymal stem/multipotent stromal cells (MSCs). *Clin Pharmacol Ther* 2007;82:241-243.
41. Summer R, Fitzsimmons K, Dwyer D, Murphy J, Fine A. Isolation of an adult mouse lung mesenchymal progenitor cell population. *Am J Respir Cell Mol Biol* 2007;37:152-159.
42. Johnson PR, Roth M, Tamm M, Hughes M, Ge Q, King G, Burgess JK, Black JL. Airway smooth muscle cell proliferation is increased in asthma. *Am J Respir Crit Care Med* 2001;164:474-477.
43. Peister A, Mellad JA, Larson BL, Hall BM, Gibson LF, Prockop DJ. Adult stem cells from bone marrow (MSCs) isolated from different strains of inbred mice vary in surface epitopes, rates of proliferation, and differentiation potential. *Blood* 2004;103:1662-1668.
44. Sacchetti B, Funari A, Michienzi S, Di CS, Piersanti S, Saggio I, Tagliafico E, Ferrari S, Robey PG, Riminucci M, et al. Self-renewing osteoprogenitors in bone marrow sinusoids can organize a hematopoietic microenvironment. *Cell* 2007;131:324-336.
45. Traktuev DO, Merfeld-Clauss S, Li J, Kolonin M, Arap W, Pasqualini R, Johnstone BH, March KL. A population of multipotent CD34-positive adipose stromal cells share pericyte and mesenchymal surface markers, reside in a periendothelial location, and stabilize endothelial networks. *Circ Res* 2008;102:77-85.