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REVIEW

Mesenchymal stromal cells: misconceptions and evolving concepts

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Abstract

Nearly half a century has passed since the publication of the first articles describing plastic-adherent cells from bone marrow, referred to initially as colony-forming unit fibroblasts, then marrow stromal cells, mesenchymal stem cells and most recently multipotent mesenchymal stromal cells (MSCs). As expected, our understanding of the nature and biologic functions of MSCs has undergone major paradigm shifts over this time. Despite significant advances made in deciphering their complex biology and therapeutic potential in both experimental animal models and human clinical trials, numerous misconceptions regarding the nature and function of MSCs have persisted in the field. Continued propagation of these misconceptions in some cases may significantly impede the advancement of MSC-based therapies in clinical medicine. We have identified six prevalent misconceptions about MSCs that we believe affect the field, and we attempt to rectify them based on current available data.

Key Words: *adult stem cells, marrow stromal cells, mesenchymal stem cells, multipotent mesenchymal stromal cells*

Introduction

Over the past several decades, concepts regarding the nature and function of mesenchymal stromal cells (MSCs) have undergone numerous major paradigm shifts. Pioneering studies by Friedenstein and colleagues first revealed that MSCs were capable of sustaining hematopoiesis and functioned as progenitors of adipogenic, chondrogenic and osteogenic lineages, properties exploited in early clinical trials (1–3). As interest in MSCs expanded, studies conducted in experimental animal models revealed the cells also possessed potent tissue reparative properties. Initial studies attributed this activity to direct cell replacement via the transdifferentiation of transplanted MSCs. However, subsequent work by many laboratories revealed that MSCs promote tissue repair via paracrine action. In recent years, the therapeutic potency of MSCs has been attributed to the secretion by cells of a large number of factors that possess angiogenic, trophic, neuro-regulatory, immunomodulatory, and anti-inflammatory activity. However, as concepts became outmoded and replaced with new paradigms, many misconceptions related to the nature and biology of MSCs arose. In this article, we identify at least six misconceptions (Figure 1) that have persisted over the years and serve as potential impediments to the successful therapeutic application of MSCs. Where possible, we attempt to clarify these misconceptions based on available published literature.

Misconceptions about MSCs

MSCs isolated from different tissues are equivalent

Although initially isolated from bone marrow (4) and then adipose tissue (5), MSCs or MSC-like cells have been identified in many tissues and organs. The apparent ubiquitous presence of MSCs in most tissues is attributed to their similarity to peri-vascular cells *in vivo*. This concept originated from studies demonstrating that bone marrow-derived MSCs express antigens common to endothelial cells and pericytes, such as STRO1 (6), CD146 and 3G5 (7), and conversely that post-capillary venule pericytes from bone marrow (6) and peri-vascular cells in most blood vessels exhibit MSC-like characteristics (7–9).

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Box 1

Misconceptions about the nature and biology of MSCs

1. MSCs isolated from different tissues are equivalent.
2. MSCs can be defined by their surface epitopes.
3. Cloning MSCs provides homogeneous preparations of cells.
4. Mouse MSCs can be isolated and expanded under the same conditions as human MSCs.
5. The properties of MSCs in culture reflect their properties *in vivo*.
6. MSCs should not be tested in clinical trials until their mechanism of action to produce therapeutically beneficial effects are fully defined.

Figure 1. General misconceptions relating to MSCs.

Subsequent studies have shown that peri-vascular cells, pericytes and fibroblasts from different tissues closely resemble the surface phenotype of MSCs, exhibit similar genome-wide expression profiles based on cluster analysis of microarray data and share similar functional properties based on qualitative *in vitro* assays (9,10). Nevertheless, closer scrutiny of these data reveal marked differences in expression levels of lineage-restricted messenger RNAs between pericytes and MSCs (10), and more rigorous *in vivo* assays demonstrate clear differences in function between cells from different tissues (11,12). For example, MSCs in general lack the contractility of pericytes, and in one study ectopic transplantation of bone marrow-derived MSCs yielded heterotopic bone tissue, whereas dental pulp-derived MSCs produced dentin and pulp tissue (12). Similarly, the capacity to generate bone and cartilage is weaker for placental and adipose-derived MSCs compared with bone marrow-derived MSCs, and the contribution to muscle fiber formation *in vivo* is greater with post-natal skeletal muscle pericytes than bone marrow-derived MSCs (13).

Several laboratories have demonstrated a neuro-ectodermal origin for MSCs. For example, Takashima *et al.* (14) demonstrated that $Sox1^{+}$ neuro-epithelial cells via a neural crest intermediate give rise to mesenchymal derivatives with properties of MSCs. Similarly, Mendez-Ferrer *et al.* (15) demonstrated that cells isolated based on expression of the neuro-epithelial marker Nestin are precursors of MSCs and can serially regenerate heterotopic osseous tissue *in vivo*. Generation of MSCs from the neural crest likely occurs via an epithelial-to-mesenchymal transition. Forced expression of the potent epithelial-to-mesenchymal transition inducer TWIST in mammary epithelial cells generated mesenchymal derivatives with MSC-like properties (16). Because TWIST plays an important role as a self-maintenance factor in MSCs (17), it may represent a useful "marker" of MSC origin and function. A neuro-ectodermal origin may also explain early results indicating that MSCs share specific traits with neural cell lineages (18). Pericytes within brain (19), thymus (20) and heart tissue (21) also reportedly derive from neural crest derivatives. These findings may explain why in some tissues MSCs and pericytes exhibit similar phenotypic and gene expression profiles.

The prevailing evidence suggests that MSCs (and pericytes) originate from several distinct developmental programs and progenitor cells. Although MSCs from different tissues share similarities in phenotypes and gene expression profiles, differences in function may be distinguished experimentally, provided that the assays are sufficiently rigorous. Consequently, not all MSCs are equivalent, and the functional attributes of populations isolated from different tissues should be carefully evaluated before implementation in clinical therapy.

MSCs are defined by their surface epitopes

Many laboratories have devoted much effort over the years to identify antigens that associate the developmental potential of MSCs with a specific phenotypic trait. MSCs express a large complement of integrin receptors (CD29, CD49a through CD49f, CD51), adhesion molecules (CD44, CD105, CD106, CD146, CD166), enzymes (CD39, CD73), growth factor receptors (CD140b, CD271, CD340, CD349), intermediate filaments (vimentin, nestin, desmin, neurofilament) and embryonic antigens (SSEA1, SSEA4), but no single molecule uniquely defines the population. Prospective isolation of MSCs with antibodies against STRO1 (6), CD271 (22) or CD146 (23), or selection for nestin-expressing cells (15) all yield the entire complement of colony-forming unit fibroblasts from marrow. Most of these antigens identify MSCs but not uniquely. Consistent with this result, analysis by a European consortium identified a complement of 113 transcripts and 17 proteins that distinguished MSCs from hematopoietic, endothelial and periosteal cells and synovial fibroblasts (24). Although the MSC committee of the International Society for Cell Therapy (25) stated in 2006 that human "MSCs must express CD105, CD73 and CD90, and lack expression of CD45, CD34, CD14 or CD11b, CD79α or CD19 and HLA-DR surface molecules," at the time this definition was recognized as being limited because the epitopes CD105, CD73 and CD90 are expressed on many different cells. The problem of ascribing a surface phenotype to MSCs is confounded further by the fact that populations exhibit significant donor-to-donor and intra-population heterogeneity (see later) and radically alter their features as they are expanded in culture (23,26,27) and after

they are administered *in vivo* (28,29). This inherent "plasticity" is thought to be key to most of the beneficial effects of MSCs in animal disease models and human clinical trials. Despite continuing efforts to identify MSC-specific epitopes, greater success may come from identifying a subset of epitopes or combinations of known markers that delineate functional differences between populations. For example, fractionation via sorting for expression of CD271, W8B2 and CD56 discriminates MSCs with chondrogenic versus adipogenic potential (30).

Cloning MSCs provides homogeneous preparations of cells

MSC populations are well known to exhibit significant donor-to-donor heterogeneity in terms of morphologic features, growth rate, differentiation potential, and potency in functional-based assays (31). These differences have been attributed to effects of donor age (32), sampling bias of marrow aspiration (33) and methods used for culture expansion of cells (34). Most of these variables can be adequately controlled, but more recent clonal-based studies have confirmed that individual populations are intrinsically heterogeneous. For example, initial studies aimed at qualifying the tri-lineage differentiation potential of MSC clones identified tri-potent, osteochondrogenic and osteogenic progenitors (35). Russell *et al.* (36) used a high-capacity, quantitative assay to measure the tri-lineage differentiation potential of human MSC clones and identified progenitors of all eight possible categories of tri-lineage potential. Subsequent studies demonstrated that clones retained their potency after amplification, and the intrinsic rate of apoptosis was significantly higher in uni-potent versus bi-potent and tri-potent clones (37). These data indicate that tri-lineage differentiation potential is intrinsically fated and specified hierarchically within populations. Reverse transcriptase polymerase chain reaction analysis further revealed a significant degree of clonal restriction in expressed levels of transcripts encoding transcription factors, signaling molecules and immunosuppressive and anti-inflammatory factors, so the extent of functional heterogeneity within populations may be large (DG Phinney, personal communication, 2012). This conclusion is further supported by computational models showing that variations in the growth rates of secondary colonies established from primary human MSC clones result from the fact that populations are hierarchically structured (38).

Although these studies demonstrate that MSC populations are a mixture of clonal progenitors of varying potency, clonal selection should generate a homogeneous cell population. Although MSCs are easier to clone than most vertebrate cells, the process of cloning does not generate homogeneous preparations. For example, 90% of the cells from some MSC preparations form single cell-derived colonies. However, as the colonies form, the morphologic features of the cells in the inner and outer regions begin to differ and this stratification is accompanied by changes in the transcriptomes of the inner and outer cells (39). In effect, MSCs in the colonies begin to generate their own microenvironment or niche. In confluent cultures, MSCs appear more homogeneous, but such cultures probably obscure much of the heterogeneity of the cells.

Mouse MSCs can be isolated and expanded under the same conditions as human MSCs

Although protocols have been developed for large-scale expansion of human MSCs (26,40), the preparation of primary MSCs from mouse bone marrow has been more challenging because of species-specific differences in the growth and adherent properties of bone marrow cells. We, and others, re-discovered a phenomenon observed many decades ago in the first attempts to culture mouse MSCs (41): mouse MSCs grow poorly when first plated in culture, then pass through a "crisis" during which most cells die but the few that survive enter a rapid growth phase. The emergence of rapidly dividing subpopulations after long-term expansion is indicative of cell immortalization, which occurs at a much higher frequency in rodent than human cells because of differences in checkpoint control mechanisms (42). Continued long-term culture of these immortalized cells leads to their transformation, as evidenced by their capacity to form tumors in mice (43). Many publications, even in high-profile journals, have used extensively expanded mouse MSCs and ignored the likelihood that the cells were immortalized. An effective protocol to isolate murine MSCs was to select them negatively with antibodies to hematopoietic cells and use the cells without expansion in culture (41). One of our laboratories more recently showed that the poor growth of primary mouse MSCs is due to oxidative stress induced by exposure to atmospheric oxygen and that oxygen-induced growth inhibition is p53-dependent (44). p53 is mutated in most immortalized rodent cell lines. Long-term exposure of mouse MSCs to atmospheric oxygen selects for clones with reduced or absent p53 function, which allows for escape from oxygen-induced growth inhibition. Many studies conducted using mouse MSCs most likely employed immortalized populations that were clonally selected. In some cases, outcomes from these studies, particularly studies comparing differences between mouse MSCs and other species, may require re-evaluation. Consequently, researchers should

stipulate whether mouse MSCs used in a given study represent primary or immortalized populations.

Properties of MSCs in culture reflect their properties in vivo

Characterization of MSCs in tissue culture initially overlooked the capacity of cells to respond dynamically to alterations in microenvironments *in vivo* produced by tissue injury, inflammation, malignant transformation and cell death. MSCs engage in cross-talk, whereby signals from target cells of the host alter the MSCs, which respond with signals that alter the target cells. MSCs *in vivo* exhibit a level of "plasticity" that is not immediately apparent or easily replicated in cells cultured *in vitro*.

One of the first examples of cross-talk was seen in co-cultures of MSCs and myeloma cells (45). Signals from myeloma cells stimulated MSCs to increase secretion of IL-6, which increased the proliferation of myeloma cells. At the same time, myeloma cells secreted high levels of Dkk-1, an inhibitor of Wnt signaling, which kept the MSCs in the cell cycle and inhibited differentiation into osteoblasts. The cross-talk provided an explanation why patients with multiple myeloma show osteolytic lesions wherein cancer cells proliferate but osteoblasts are not recruited to fill the lesions (46). Similar examples of cross-talk were observed in experiments of mouse MSCs infused after sepsis induced by cecal ligation and puncture (28) and in the experimental autoimmune encephalomyelitis model of multiple sclerosis (47). In some cases, MSCs up-regulated the expression of several hundred genes that were minimally expressed or not expressed in culture. The up-regulated genes and factors included nitric oxide (48), indoleamine 2,3-dioxygenase (49), prostaglandin E_2 (28), and the anti-inflammatory protein TSG-6 (27). This cellular cross-talk may explain why recent outcomes in MSC-based clinical trials to treat graft-versus-host disease were not predicted based on the immunosuppressive activity of donor MSCs evaluated *in vitro* (50). These results stress the need for caution when extrapolating results from *in vitro* studies to mechanism of action *in vivo* because the two may not always be congruent.

MSC-based clinical trials are unwarranted until the molecular mechanisms by which the cells produce their beneficial effects are defined

Although more opinion than misconception, it seems prudent to discuss the benefits of clinical studies in determining whether results obtained in experimental animal studies can be translated into human patients. Clinical trials have a long history of advancing medical science. Most notable is the history of bone marrow transplantation, the first attempts of which failed because of lack of knowledge regarding the existence and function of the major histocompatibility complex. However, owing to the perseverance of early pioneers in the field, bone marrow transplantation is now performed routinely to treat a wide variety of disease indications. The fact that numerous MSC-based clinical trials are currently in progress despite lack of specific knowledge regarding the mechanism of action of cells *in vivo* is consistent with this history. Although early clinical trials using MSCs were not definitive (2,3), they demonstrated a lack of adverse effects associated with intravenous infusions of large numbers of cells into patients, which has fueled the rapid expansion of MSC-based trials in clinical medicine. However, obtaining definitive data on the effectiveness of MSCs in the clinic remains problematic for several reasons. One challenge is that different protocols are being used to prepare the MSCs, and we lack useful tests to compare their potency. The biologic properties of MSCs used in different clinical trials are difficult to compare, and in some cases, patients may receive cells that vary considerably in their composition and function. A second problem is that many trial outcomes fail to demonstrate a dose-dependent effect of MSCs in patients; this raises serious concerns that the mechanism of action of cells is not well defined *in vivo*. Finally, scrutiny of clinical outcome data is hampered by the fact in some cases data from industry-sponsored trials are not published, and outcomes from patients administered MSCs in a hospital setting as a practice of medicine are not recorded within national or international databases. Efforts to establish and maintain registries of MSC-based therapies should be expanded. Despite documented evidence that some patients benefit from MSC-based therapies, many challenges remain before definitive conclusions can be made regarding the overall efficacy of MSC-based therapies.

Conclusions

The relative ease by which MSCs can be harvested and expanded to large numbers *in vitro*, coupled with their potent trophic, anti-inflammatory and immunomodulatory activity and lack of infusion-related toxicity in human patients has made MSCs an attractive tool for cellular therapy; this is reflected by the rapid increase in the number of ongoing MSC-based clinical trials. However, as our knowledge regarding the complex biology of MSC increases, it is necessary to discard outmoded concepts and misconceptions about these cells, particularly those that may delay advancements in the field. For

example, initial studies demonstrating that MSC populations from different tissue sources or human donors are homogeneous and functionally equivalent based on analysis of surface phenotype are slowly being supplanted by functional studies that demonstrate intrinsic intra-population heterogeneity. These results have important implications with respect to large-scale expansion of MSCs for clinical use. Consequently, continued research focused on basic MSC biology will continue to be important for advancing cellular therapies in human patients.

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References

1. Phinney DG. Marrow stem. In: Polak J, Mantalaris S, Harding S, editors. Advances in tissue engineering. London: Imperial College Press; 2008. p. 95–122.
2. Horwitz EM, Prockop DJ, Fitzpatrick LA, Koo WW, Gordon PL, Neel M, et al. Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. Nat Med. 1999;5:309–13.
3. Koc O, Gerson SL, Cooper BW, Dyhouse SM, Haynesworth SE, Caplan AI, et al. Rapid hematopoietic recovery after coinfusion of autologous-blood stem cells and culture-expanded marrow mesenchymal stem cells in advanced breast cancer patients receiving high-dose chemotherapy. J Clin Oncol. 2000;18:307–16.
4. Friedenstein AJ, Gorskaja JF, Kulagina NN. Fibroblast precursors in normal and irradiated mouse hematopoietic organs. Exp Hematol. 1976;4:267–74.
5. Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, et al. Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell. 2002;13:4279–95.
6. Gronthos S, Graves SE, Ohta S, Simmons PJ. The STRO1+ fraction of adult human bone marrow contains the osteogenic precursors. Blood. 1994;84:4164–73.
7. Shi S, Gronthos S. Perivascular niche of postnatal mesenchymal stem cells in human bone marrow and dental pulp. J Bone Min Res. 2003;18:694–704.
8. Brachvogel B, Moch H, Pausch F, Schlotzer-Schrehardt U, Hofmann C, von der Mark K, et al. Perivascular cells expressing annexin A5 define a novel mesenchymal stem cell-like population with the capacity to differentiate into multiple mesenchymal lineages. Development. 2005;132:2657–68.
9. Crisan M, Yap S, Casteilla L, Chen CW, Corselli M, Park TS, et al. A perivascular origin for mesenchymal stem cells in multiple human organs. Cell Stem Cell. 2008;3:301–13.
10. Covas DT, Panepucci RA, Fontes AM, Silva WA Jr, Orellana MD, Freitas MC, et al. Multipotent mesenchymal stromal cells obtained from diverse human tissues share functional properties and gene-expression profile with CD146+ perivascular cells and fibroblasts. Expt Hematol. 2008;36:642–54.
11. da Silva Meirelles L, Chagastelles PC, Nardi NB. Mesenchymal stem cells reside in virtually all post-natal organs and tissues. J Cell Sci. 2006;119:2204–13.
12. Batouli S, Miura M, Brahim J, Tsutsui TW, Fisher LW, Gronthos S, et al. Comparison of stem-cell-mediated osteogenesis and dentinogenesis. J Dent Res. 2003;2:976–81.
13. Dellavalle A, Maroli G, Covarello D, Azzoni E, Innocenzi A, Perani L, et al. Pericytes resident inpostnatal skeletal muscle differentiate into muscle fibres and generate satellite cells. Nat Comm. 2011;2:499.
14. Takashima Y, Era T, Nakao K, Kondo S, Kasuga M, Smith AG, et al. Neuroepithelial cells supply an initial transient wave of MSC differentiation. Cell. 2007;129:1377–88.
15. Mendez-Ferrer S, Michurina TV, Ferraro F, Mazloom AR, Macarthur BD, Lira SA, et al. Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. Nature. 2010;466:829–34.
16. Battula VL, Evans KW, Hollier BG, Shi Y, Marini FC, Ayyanan A, et al. Epithelial-mesenchymal transition-derived cells exhibit multi-lineage differentiation potential similar to mesenchymal stem cells. Stem Cells. 2010;28:1435–45.
17. Isenmann S, Arthur A, Zannettino AC, Turner JL, Shi S, Glackin CA, et al. TWIST family of basic helix-loop-helix transcription factors mediate human mesenchymal stem cell growth and commitment. Stem Cells. 2009;27:2457–68.
18. 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; 25:2896–902.
19. Etchevers HC, Vincent C, Le Douarin NM, Couly GF. The cephalic neural crest provides pericytes and smooth muscle cells to all blood vessels of the face and forebrain. Development. 2001;128:1059–68.
20. Muller SM, Stolt CC, Terszowski G, Blum C, Amagai T, Kessaris N, et al. Neural crest origin of perivascular mesenchyme in the adult thymus. J Immunol. 2008;180:5344–51.
21. Brown CB, Baldwin HS. Neural crest contribution to the cardiovascular system. Adv Exp Med Biol. 2006;589:134–54.
22. Quirici N, Soligo D, Bossolasco P, Servida F, Lumini C, Deliliers GL. Isolation of bone marrow mesenchymal stem cells by anti-nerve growth factor receptor antibodies. Exp Hematol. 2002;30:783–91.
23. Sacchetti B, Funari A, Michienzi S, Di Cesare S, Piersanti S, Saggio I, et al. Self-renewing osteoprogenitors in bone marrow sinusoids can organize a hematopoietic microenvironment. Cell. 2007;131:324–36.
24. Charbord P, Livne E, Gross G, Haupl T, Neves NM, Marie P, et al. Human bone marrow mesenchymal stem cells: a systematic reappraisal via the genostem experience. Stem Cell Rev. 2011;7:32–42.
25. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy. 2006;8:315–7.
26. 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;107:275–81.

27. Larson BL, Ylöstalo J, Prockop DJ. Human multipotent stromal cells undergosharp transition from division to development in culture. Stem Cells. 2008;26:193–201.
28. Németh K, Leelahavanichkul A, Yuen PS, Mayer B, Parmelee A, Doi K, et al. Bone marrow stromal cells attenuate sepsis via prostaglandin E(2)-dependent reprogramming of host macrophages to increase their interleukin-10 production. Nat Med. 2009;15:42–9.
29. Lee RH, Pulin AA, Seo MJ, Kota DJ, Ylostalo J, Larson BL, et al. Intravenous hMSCs improve myocardial infarction in mice because cells embolized in lung are activated to secrete the anti-inflammatory protein TSG-6. Cell Stem Cell. 2009;5: 54–63.
30. Battula VL, Treml S, Bareiss PM, Gieseke F, Roelofs H, de Zwart P, et al. Isolation of functionally distinct mesenchymal stem cell subsets using antibodies against CD56, CD271, and mesenchymal stem cell antigen 1. Haematologica. 2009;94:173–84.
31. Phinney DG. Biochemical heterogeneity of mesenchymal stem cell populations: clues to their therapeutic efficacy. Cell Cycle. 2007;6:2884–9.
32. Zhou S, Greenberger JS, Epperly MW, Goff JP, Adler C, Leboff MS, et al. Age-related intrinsic changes in human bone-marrow-derived mesenchymal stem cells and their differentiation to osteoblasts. Aging Cell. 2008;7:335–43.
33. Muschler GF, Boehm C, Easley K. Aspiration to obtain osteoblast progenitor cells from human bone marrow: the influence of aspiration volume. J Bone Joint Surg Am. 1997; 79:1699–709.
34. Wagner W, Ho AD. Mesenchymal stem cell preparations—comparing apples and oranges. Stem Cell Rev. 2007;3:239–48.
35. Muraglia 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: 1161–6.
36. Russell KC, Phinney DG, Lacey MR, Barrilleaux BL, Meyertholen KE, O'Connor KC. In vitro high-capacity assay to quantify the clonal heterogeneity in trilineage potential of mesenchymal stem cells reveals a complex hierarchy of lineage commitment. Stem Cells. 2010;28:788–98.
37. Russell KC, Lacey MR, Gilliam JK, Tucker HA, Phinney DG, O'Connor KC. Clonal analysis of the proliferation potential of human bone marrow mesenchymal stem cells as a function of potency. Biotechnol Bioeng. 2011;108: 2716–26.
38. Sengers BG, Dawson JI, Oreffo ROC. Characterization of human bone marrow stromal cell heterogeneity for skeletal regeneration strategies using a two-stage colony assay and computational modeling. Bone. 2010;46:496–503.
39. Ylöstalo J, Bazhanov N, Prockop DJ. Reversible commitment to differentiation by human multipotent stromal cells in single-cell-derived colonies. Exp Hematol. 2008;36:1390–402.
40. Sotiropoulou PA, Perez SA, Salagianni M, Baxevanis CN, Papamichail M. Characterization of the optimal culture conditions for production of human mesenchymal stem cells. Stem Cells. 2006;24:42–7.
41. Phinney DG. Isolation of mesenchymal stem cells from murine bone marrow by immunodepletion. Methods Mol Biol. 2008;449:171–86.
42. Prowse KR, Greider CW. Developmental and tissue-specific regulation of mouse telomerase and telomere length. Proc Natl Acad Sci U S A. 1995;92:4818–22.
43. Tolar J, Nauta AJ, Osborn MJ, Panoskaltsis Mortari A, McElmurry RT, Bell S, et al. Sarcoma derived from cultured mesenchymal stem cells. Stem Cells. 2007;25:371–9.
44. Boregowda S, Krishnappa V, Chambers J, LoGrasso PV, Lai W-T, Ortiz LA, et al. Atmospheric oxygen inhibits growth and differentiation of marrow-derived mouse mesenchymal stem cells via a p53 dependent mechanism: implications for procurement methods that rely on long-term culture expansion of bone marrow. Stem Cells. 2012;30: 975–87.
45. Gunn WG, Conley A, Deininger L, Olson SD, Prockop DJ, Gregory CA. A crosstalk between myeloma cells and marrow stromal cells stimulates production of DKK1 and interleukin-6: a potential role in the development of lytic bone disease and tumor progression in multiple myeloma. Stem Cells. 2006;24: 986–91.
46. Qiang YW, Barlogie B, Rudikoff S, Shaughnessy JD Jr. Dkk1-induced inhibitionof Wnt signaling in osteoblast differentiation is an underlying mechanism of boneloss in multiple myeloma. Bone. 2008;42:669–80.
47. Zappia E, Casazza S, Pedemonte E, Benvenuto F, Bonanni I, Gerdoni E, et al. Mesenchymal stem cells ameliorate experimental autoimmune encephalomyelitis inducing T-cell anergy. Blood. 2005;106:1755–61.
48. Ren G, Zhang L, Zhao X, Xu G, Zhang Y, Roberts AI, et al. Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. Cell Stem Cell. 2008;2:141–50.
49. François M, Romieu-Mourez R, Li M, Galipeau J. Human MSC suppression correlates with cytokine induction of indoleamine 2,3-dioxygenase and bystander M2 macrophage differentiation. Mol Ther. 2012;20:187–95.
50. Le Blanc K, Frassoni F, Ball L, Locatelli F, Roelofs H, Lewis I, et al. Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study. Lancet. 2008;371:1579–86.