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Building a Consensus Regarding the Nature and Origin of Mesenchymal Stem Cells

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Abstract Mesenchymal stem cells (MSCs) are believed to be the common precursors to differentiated cell lineages found in bone and bone marrow, including adipocytes, chondrocytes, osteoblasts, and hematopoiesis-supporting stroma. Apart from this fact, most aspects of MSC biology, including their ontogeny, anatomical location in marrow, and in vivo functions remain vague. Attempts to clarify these issues have produced confounding results, principally due to the fact that many researchers employ different methods to culture MSCs, assess their differentiation potential, and evaluate their capacity for self-renewal. Accordingly, the current status of the field appears fragmentary with no clear consensus on how to define the cells. In describing past and present contributions to the field of MSC research, I will demonstrate that the apparent incongruity of the literature is misleading, and that an unbiased interpretation reveals a fairly cohesive picture of MSC biology. J. Cell. Biochem. Suppl. 38: 7–12, 2002.

Key words: marrow stromal cells; osteogenic stem cells; pericytes

Research spanning over 40 years has contributed to the discovery in bone marrow of a cell population that are precursors of connective tissue cell types, including adipocytes, chondrocytes, osteoblasts, and hematopoiesis-supporting stroma. These cells have been denoted in the past literature by a variety of monikers, including fibroblast colony-forming cells, colony-forming unit-fibroblasts, mesenchymal progenitor cells, or marrow stromal cells. Currently, they are referred to as mesenchymal stem cells (MSCs), although it remains in question whether they are bona fide stem cells or determined progenitors of connective tissues. This debate is due, in part, to the lack of suitable assays whereby properties of the MSC, in particular its capacity for self-renewal, can be accurately assessed. It also results from the fact that the methods used to culture MSCs produce a heterogeneous cell population that exhibits variable characteristics with regard to phenotype and function. Accordingly, there is a lack of consensus on how to define MSCs.

In spite of the contentious nature of the field, interest in MSCs continues to grow, particularly with regard to their therapeutic potential. Currently, MSCs are being evaluated as therapeutic agents in animal models of paralysis, stroke, heart attack, and neurodegenerative disease as well as in human clinical trials for osteogenesis imperfecta. However, due to their indeterminate nature, it is difficult to assess the number and quality of actual stem cells being evaluated in these studies. Biased sample preparations may result in an inaccurate assessment of the overall therapeutic potential of MSCs. Because such studies will largely determine their clinical relevance as agents to treat disease, the necessity of developing standardized methods to isolate, phenotype, and evaluate the quality of MSCs is ever increasing. Accordingly, by distilling pertinent aspects of the past and present literature, this review will attempt to build a consensus regarding the nature and origin of MSCs.

FRIEDENSTEIN, OSTEOGENIC STEM CELLS, AND MSCS

Alexander Friedenstein is generally credited with the discovery of MSCs. However, the scope

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and significance of his work is often understated, principally with regard to describing key aspects of MSC biology, including their capacity for self-renewal in vivo. Therefore, in building a consensus regarding the nature and origin of MSCs, it seems appropriate to begin by restating his contributions to the field.

Nearly 40 years ago, Petrakova et al. [1963] demonstrated that pieces of bone marrow transplanted under the renal capsule of mice formed an osseous tissue over a period of several weeks that was invaded by hematopoietic cells. Alternatively, when marrow cells were transplanted in a closed system, i.e., a diffusion chamber, the cells also formed bone tissue but colonization by hematopoietic cells did not occur [Friedenstein et al., 1966]. These early studies revealed that bone marrow was inherently osteogenic, a feature that distinguished it from other tissues such as transitional epithelium [Friedenstein, 1961], and suggested that osteogenic precursors in marrow were distinct from those that gave rise to hematopoietic cells.

To demonstrate that bone marrow contained separable populations of osteogenic and hematopoietic precursors, Friedenstein et al. [1968] transplanted mouse marrow cells from a parental strain under the renal capsule of F_1 hybrids (semi-syngeneic). Karyotype analysis revealed that the resulting bone tissue formed was genetically identical to the parental strain but the hematopoietic tissue that colonized it was of host origin. These results were confirmed by showing that the ossicle continued to form new bone when transplanted back into the parental strain but the hematopoietic cells within it were rejected. Conversely, when male marrow cells recovered from a heterotopic ossicle were transplanted into syngeneic females immunized against male antigens, the osteogenic cells were rejected by the host immune system and no bone tissue was formed [Friedenstein and Kuralesova, 1971]. These studies confirmed that osteogenic and hematopoietic cells were derived from distinct precursors in marrow. They also demonstrated that heterotopic osseous tissue was self-maintaining, i.e., it was not replaced or remodeled by cells from the host animal, even after prolonged periods of time in vivo. These authors subsequently showed that marrow cells were capable of continually regenerating new osseous tissue when serially transplanted to secondary recipients, but that their osteogenic capacity was eventually exhausted by the 5th passage. Therefore, the osteogenic cells exhibited a capacity for self-maintenance commensurate with that demonstrated for hematopoietic stem cells passaged through irradiated recipients. These results led to the classification of the cells as osteogenic stem cells.

To identify the nature of the osteogenic stem cell, Friedenstein et al. [1970] developed a simple method to isolate fibroblastoid (stromal) cells from bone marrow based on their adherence to tissue culture plastic, a method still widely employed today due to its effectiveness. Friedenstein demonstrated that these stromal cells were (1) a rare population in bone marrow, (2) did not enter S phase until up to 60 h after initial plating, (3) had a high replicative capacity in vitro, (4) were clonegenic, and (5) formed colonies of irregular shape and density. More importantly, he showed the cells were capable of forming bone tissue in vivo, even after multiple passages in vitro, and hence were a source of osteogenic stem cells and/or their progeny.

Collectively, these studies demonstrated that bone marrow contains a cell population, distinct from hematopoietic lineages, that is capable of protracted self-maintenance and differentiation into multiple mesenchymal cell lineages. Accordingly, the cells fulfill the criteria of MSCs. Therefore, the most pertinent question in the field today is not whether MSCs exist, but rather how to define them.

HETEROGENEITY OF MARROW STROMAL CELL CULTURES

One obstacle in defining MSCs is the lack of suitable conditions for their maintenance and expansion ex vivo from an adherent population of marrow fibroblasts (stromal cells) that is morphologically, phenotypically, and functionally heterogeneous. Friedenstein first noted that cultured stromal cell populations varied in size, morphology, proliferative potential, expressed varying levels of alkaline phosphatase, and possessed different osteogenic capacities in vivo [Friedenstein et al., 1982]. Subsequently, various groups demonstrated that marrow stromal cells were capable of multi-lineage differentiation in vitro [Beresford et al., 1992; Umezawa et al., 1992; Dorheim et al., 1993; Dennis et al., 1999] but these and other studies made it apparent that both clonal and nonclonal populations were functionally heteroge-

neous [Kuznetsov et al., 1997; Phinney et al., 1999]. Recently, Mauraglia et al. [2000] showed that of 185 non-immortalized human marrow stromal cell clones evaluated, approximately half expressed osteo-chondrogenic potential and less than one-third were capable of differentiating into bone, fat, and cartilage. More importantly, the multi-potential clones progressively lost their adipogenic and chondrogenic potential at increasing passage number. This result is consistent with a previous report demonstrating a loss of multi-potentiality in human marrow stromal cells as they were culture-expanded [DiGirolamo et al., 1999]. Collectively, these findings indicate that stromal cell cultures are an admixture of cells with varying developmental potentials and that culture conditions used to expand the cells in vitro do not support long-term self-maintenance of the MSC.

Recently, Pittenger et al. [1999] described conditions for isolation and culture-expansion of a homogenous population of human MSCs. Homogeneity in this case was defined by uniform expression of approximately 50 distinct antigens by the cells and their essentially complete differentiation into three separate lineages; bone, fat, and cartilage. Methods employed by these authors differed from the aforementioned studies in that cells were cultivated in sera lots pre-screened using a rigorous battery of tests. This difference is significant in that batches of sera are typically poorly characterized and inconsistent from batch to batch, and as a result can have dramatically different affects on cell attachment, spreading, growth, and differentiation [Barnes and Sato, 1980]. For example, Lennon et al. [1996] demonstrated that human marrow stromal cells cultured in different sera batches exhibited growth rates in vitro that varied up to 6-fold. Moreover, only 3 out of 10 sera lots tested supported in vivo osteogenic differentiation of the cells. Batch to batch variations in sera were also shown to affect the differentiation potential of stage 24 embryonic chick limb bud mesenchymal cells.

Although the selection of sera lots as described above likely provides a more suitable microenvironment for expansion of stem/progenitor cells, this adaptation alone is not sufficient for long-term maintenance of the MSC. This is indicated by the fact that the cells cultured in pre-screened sera lots exhibited donor-to-donor variability in growth rate at late passage, together with loss of chondrogenic potential beyond passage 5, and decreased osteogenic potential beyond passage 8 [Pittenger et al., 2001]. Notably, the loss of multi-potentiality in these cultures was not discernable by a change in phenotype. Therefore, the antigens used to measure homogeneity failed to reflect the developmental potential of the cells.

MOLECULAR CHARACTERIZATION OF MSCS

The heterogeneous nature of stromal cell cultures has confounded efforts to characterize the molecular phenotype of the MSC. In addition to their functional heterogeneity, the cultures display varied morphologies and express lineage-specific antigens that can vary between different preparations and as a function of time in culture [Charbord et al., 1990; Perkins and Fleishman, 1990]. Many past characterizations of the cells have principally involved analysis of one or a few expressed antigens. To provide a more comprehensive molecular description of the cells, my laboratory used serial analysis of gene expression (SAGE) to catalog 2,353 expressed transcripts in a single cell-derived colony of human stromal cells elaborated from low density cultures [Tremain et al., 2001]. This analysis revealed the colony simultaneously expressed mRNAs of different mesenchymal cell lineages, including chondrocytes, myoblasts, osteoblasts, and hematopoiesis-supporting stroma as well as various transcripts characteristic of endothelial, epithelial, and neural cells. However, many of the cytokines, cytokine receptors, integrins, and matrix molecules reportedly expressed in stromal cells [Haynesworth et al., 1996; Pittenger et al., 2001] were not expressed by the single cell-derived colony. These differences suggest that a single cell-derived colony is comprised of distinct subsets of cells as compared to stromal cell cultures elaborated using standard methods, i.e., passage at high density.

Recently, human marrow stromal cells grown at low density (10 cells/cm^2) were shown to possess a significantly greater replicative potential than compared to cells passaged as monolayers [DiGirolamo et al., 1999]. These low-density cultures are enriched for a sub-population of cells, termed rapidly self-renewing (RS) cells, that can be discriminated by their differential expression of several antigens, including the TrkA, Flk-1, and c-kit receptors [Colter et al.,

2001]. The RS cells appear to be highly mitotic, which is consistent with the fact that populations cultured at low density contain a high proportion of cells in S phase. We recently compared the transcript profile of a population of stromal cells propagated for two passages at either low (6 cells/cm^2) or high (1,000 cells/cm^2) density. Surprisingly, this change in density altered the expression of over 400 separate transcripts by a factor of 3-fold or greater (C McBride, DG Phinney, unpublished results). Collectively, these results indicate that culture density affects both the biological properties and molecular phenotype of stromal cells.

ONTOGENY AND ANATOMICAL LOCATION OF MSCS

Unfortunately, no specific antigen(s) has been described that associates the developmental potential of MSCs with a specific phenotypic trait. In the absence of such a reagent, it is impossible to determine the proportion of stem cells, multi-potential progenitors, and determined precursors in cultured stromal cell populations or the anatomical location of these cells within bone marrow. Several laboratories have isolated monoclonal antibodies, including Stro-1 [Gronthos et al., 1994] and SB-10 [Bruder et al., 1997] that are immuno-reactive against stromal cells cultured in vitro. Recently, Stro-1 was shown to bind to the walls of the microvasculature in a variety of tissues [Bianco et al., 2001]. This result is not surprising in that the prominent fibroblast-like cell in bone marrow (and presumably in stromal cell cultures) is the reticular cell, which covers the abluminal surface of the endothelium of the venous sinuses and as such represents a specialized marrow pericyte [Weiss, 1976]. As the name implies, these cells display a "reticular" morphology, projecting cytoplasmic processes into the marrow sinus where they convey signals to maturing hematopoietic cells. The reticular cells also differentiate into adipocytes in situ, thereby regulating the size and permeability of the sinusoidal system in bone marrow. Several studies have shown that preformed, post-capillary venule pericytes from bone marrow are able to differentiate into osteoblasts and chondrocytes in vivo [Diaz-Flores et al., 1991]. These unique properties have led Bianco and Cossu [1999] to suggest that marrow pericytes represent the closest in vivo approximation to the MSC.

Our SAGE analysis indicates that stromal cells from post-natal marrow simultaneously express characteristics of mesenchymal, endothelial, and epithelial cells, a distinguishing feature of mesothelium. Recently, Munoz-Chapuli et al. [1999] provided evidence that cells within the splanchnic mesothelium, an epithelial lining of the coelom, detach from their neighbors, invade the adjacent splanchnic mesoderm, and undergo an epithelial-to-mesenchymal transition as evidenced by the appearance of increased mitotic figures, long cytoplasmic basal projections, and expression of the intermediate filament vimentin. This invasion of mesothelial cells occurs at about the same time as the appearance of primitive endothelial and hematopoietic progenitors within the splanchnopleura. Transient co-expression of cytokeratins, vimentin, and specific hemangioblastic markers by these progenitors suggests they are derived from mesothelial cells. The primitive endothelial cells within the splanchnopleura colonize the floor of the aorta and differentiate in situ to produce the vasculature of the body wall, kidney, visceral organs, and limbs [Pardanaud et al., 1996]. This process of vasculogenesis, therefore, is consistent with the notion that mesothelial-derived MSCs are localized to bone marrow via invasion of the vasculature and may represent a specialized type of vascular stem cell.

CONCLUSIONS

The data described within this review provides a coherent interpretation of MSC biology. Friedenstein et al. demonstrated the existence of a MSC in bone marrow. Presumably, these stem cells are delivered to this tissue during embryogenesis when the cartilaginous analge of the presumptive bone is invaded by the vasculature. During this process MSCs may be localized to the invading edge of the vasculature, generating the cell types necessary for the development of the bone and bone marrow. Alternatively, MSCs may be deposited into specialized niches within the preformed marrow and function as a repository for precursor cells needed for the growth and remodeling of the adult tissue. Based on the hierarchical model of differentiation proposed by Caplan [1994], MSCs would yield progeny that through a determined series of events differentiate into progressively more restricted precursors

of connective tissue cell types. Self-renewal and lineage commitment of MSCs may be governed by intrinsic or extrinsic factors, the nature of which cannot currently be predicted. In this scheme, the MSC would be upstream of the stromal or reticular cell, since the later represent a specialized cell type in bone marrow. Conversely, these reticulating vascular cells may be more akin to liver hepatocytes, wherein they perform specialized functions in marrow but retain the capacity to differentiate into multiple mesenchymal lineages in response to instructive signals generated during matrix remodeling of the tissue.

Following removal from the marrow microenvironment, the actual fate of the MSC is unknown. Clearly, marrow stromal cell cultures are an admixture of molecularly and functionally distinct cell types. This heterogeneity likely reflects the complexity of the marrow stroma, contributed in part by specialized sub-populations of stromal (reticular) cells that interact with different hematopoietic cells. These stromal cells also have the capacity to alter their function (and phenotype) in response to external stimuli [Miller-Sieburg and Deryugina, 1995]. Continuous growth and remodeling of bone tissue would also imply that marrow contains progenitor cells of varying developmental potential. Predictably, the actual number of MSCs in these cultures would be very few. When cultured in vitro MSCs and their progeny progressively lose potential with increasing passage number, indicating that conditions currently employed to expand the cells does not recapitulate the in vivo microenvironment that supports self-maintenance of the MSC. This problem is likely exasperated by the fact that agents in the media (or sera) instruct progenitor cells to adopt specific fates and/or selectively support expansion of determined progenitors. Loss of stem cell potential may also be due to rapid expansion and subsequent exhaustion of the stem cell pool.

Plating density also appears to affect the biological properties of marrow stromal cells. Cultures passaged at low density possess greater overall replicative potential, although this has not been directly correlated with extended lifespan of the MSC. Low cell density in culture may prevent the generation of a nutrient or oxygen deficient environment, limit the extent of cell-to-cell contact, and maintain a greater proportion of the cells in cycle, any of which may prevent cellular differentiation. In contrast, culturing stromal cells at high density likely mimics the in vivo microenvironment of developing mesodermal tissues and induces differentiation of the stem/progenitor pool. For example, condensation of presumptive mesenchyme precedes endochondrial and intramembraneous bone formation as well as the regeneration of limbs in amphibians. Plating density is also known to alter the phenotype expressed by chick limb bud mesenchymal cells in vitro [Lennon et al., 1996] and culturing MSCs at high density promotes their chondrogenic differentiation [Johnstone et al., 1998].

Obviously, the complexity of marrow stromal cell cultures has created an obstacle to defining the nature of the MSC, and has led to the suggestion that MSCs may represent a unique type of stem cells with unconventional properties. This problem is exacerbated by the fact that connective tissue derived from stroma of spleen and thymus as well as the abdominal wall of the body cavity can be induced to differentiate into bone producing cells under the appropriate conditions. Therefore, an important first step in bringing continuity to the field is developing a standardized method to evaluate the stem cell characteristics, namely self-renewal and multilineage differentiation, of putative MSC populations. Serial regeneration in vivo of a heterotopic ossicle may be the best experimental approximation of these processes and provide a means to associate stem cell characteristics with specific phenotypic traits. Defining even one ligand/receptor or signaling molecule that mediates self-renewal and/or lineage commitment in MSCs will move the field in a new direction that ultimately will delineate the validity of the mesengenic process. Only then we can begin to evaluate the true potential of the MSC.

REFERENCES

Barnes D, Sato G. 1980. Methods for growth of cells in serum-free medium. Anal Biochem 102:255–270.

Beresford JN, Bennet JH, Devlin C, Leboy PS, Owen M. 1992. Evidence for an inverse relationship between the differentiation of adipocytic and osteogenic cells in rat marrow stromal cell cultures. J Cell Sci 102:341–351.

Bianco P, Cossu G. 1999. Uno, nessuno e centomila: searching for the identity of mesodermal progenitors. Expt Cell Res 251:257–263.

Bianco P, Riminucci M, Gronthos S, Robey PG. 2001. Bone marrow stromal stem cells: nature, biology, and potential applications. Stem Cells 19:180–192.

Bruder SP, Horowitz MC, Mosca JD, Haynesworth SE. 1997. Monoclonal antibodies reactive with human osteogenic cell surface antigens. Bone 21:225–235.

Caplan AI. 1994. The mesengenic process. Clin Plast Surg 21:429–435.

Charbord P, Lerat H, Newton I, Tamayo E, Gown AM, Singer JW, Herve P. 1990. The cytoskeleton of stromal cells from human bone marrow cultures resembles that of cultured smooth muscle cells. Exp Hematol 18:276–282.

Colter DC, Sekiya I, Prockop DJ. 2001. Identification of a subpopulation of rapidly self-renewing and multipotential adult stem cells in colonies of human marrow stromal cells. Proc Natl Acad Sci USA 98:7841–7845.

Dennis JE, Merriam A, Awadallah A, Yoo JU, Johnstone B, Caplan AI. 1999. A quadripotential mesenchymal progenitor cell isolated from the marrow of an adult mouse. J Bone Miner Res 14:700–709.

Diaz-Flores L, Gutierrez R, Gonzalez P, Varela H. 1991. Inducible perivascular cells contribute to the neochondrogenesis in grafted perichondrium. Anat Rec 229:1–8.

DiGirolamo CM, Stokes D, Colter D, Phinney DG, Class R, Prockop DJ. 1999. 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. Brit J Hematol 107:275–281.

Dorheim MA, Sullivan M, Dandapani V, Wu X, Hudson J, Segarini PR, Rosen DM, Aulthouse AL, Gimble JM. 1993. Osteoblastic gene expression during adipogenesis in hematopoietic supporting murine bone marrow stromal cells. J Cell Physiol 154:317–328.

Friedenstein AJ. 1961. Osteogenetic activity of transplanted epithelium. Acta Anat 45:31–59.

Friedenstein AJ. 1962. Humoral nature of osteogenic activity of transitional epithelium. Nature 194:698–699.

Friedenstein AJ, Kuralesova AI. 1971. Osteogenic precursor cells of bone marrow in radiation chimeras. Transplantation 12:99–108.

Friedenstein AJ, Piatetzky-Shapiro II, Petrakova KV. 1966. Osteogenesis in transplants of bone marrow cells. J Embryol Exp Morph 16:381–390.

Friedenstein AJ, Petroakova KV, Kurolesova AI, Frolova GP. 1968. Heterotopic transplants of bone marrow. Transplantation 6:230–247.

Friedenstein AJ, Chailakhjan RK, Lalykina KS. 1970. The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells. Cell Tissue Kinet 3:393–403.

Friedenstein AJ, Latzinik NW, Grosheva AG, Gorskaya UF. 1982. Marrow microenvironment transfer by heterotopic transplantation of freshly isolated and cultured cells in porous sponges. Exp Hematol 10:217–227.

Gronthos S, Graves SE, Ohta S, Simmons PJ. 1994. The STRO-1 + fraction of adult human bone marrow contains the osteogenic precursors. Blood 84:4164–4173.

Haynesworth SE, Baber MA, Caplan AI. 1996. Cytokine expression by human marrow derived mesenchymal progenitor cells in vitro: effects of dexamethasone and IL-1α. J Cell Physiol 166:585–592.

Johnstone B, Hering TM, Caplan AI, Goldberg VM, Yoo JU. 1998. In vitro chondrogenesis of bone marrow-derived mesenchymal progenitor cells. Expt Cell Res 238:265–272.

Kuznetsov SA, Krebsbach PH, Satomura K, Kerr J, Riminucci M, Benayahu D, Robey PG. 1997. Single-colony derived strains of human marrow stromal fibroblasts form bone after transplantation in vivo. J Bone Miner Res 12:1335–1347.

Lennon DP, Haynesworth SE, Bruder SP, Jaiswal N, Caplan AI. 1996. Human and animal mesenchymal progenitor cells from bone marrow: identification of serum for optimal selection and proliferation. In Vitro Cell Dev Biol 32:602–611.

Mauraglia A, Cancedda R, Quarto R. 2000. Clonal mesenchymal progenitors from human bone marrow differentiate in vitro according to a hierarchical model. J Cell Sci 113:1161–1166.

Miller-Sieburg C, Deryugina E. 1995. The stromal cells' guide to the stem cell universe. Stem Cells 13:477–486.

Munoz-Chapuli R, Perez-Pomares JM, Macias D, Garcia-Garrido L, Carmona R, Gonzalez M. 1999. Differentiation of hemangioblasts from embryonic mesothelial cells? A model on the origin of the vertebrate cardiovascular system. Differentiation 64:133–141.

Pardanaud L, Luton D, Prigent M, Bourcheix L-M, Catala M, Dieterien-Lievre F. 1996. Two distinct endothelial lineages in ontogeny, one of them related to hematopoiesis. Development 122:1363–1371.

Perkins S, Fleishman RA. 1990. Stromal cell progeny of murine bone marrow fibroblast colony-forming units are clonal endothelial-like cells that express collagen IV and laminin. Blood 75:620–625.

Petrakova KV, Tolmacheva AA, Friedenstein AJ. 1963. Bone formation occurring in bone marrow transplantation in diffusion chambers. Bull Exp Biol Med 56:87–91.

Phinney DG, Kopen G, Righter W, Webster S, Tremain N, Prockop DJ. 1999. Donor variation in the growth properties and osteogenic potential of human marrow stromal cells. J Cell Biochem 75:424–436.

Pittenger MF, MacKay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. 1999. Multilineage potential of adult human mesenchymal stem cells. Science 284:143–147.

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 Britian: Kluwer Academic Publishers. p 189–207.

Tremain N, Korkko J, Ibberson D, Kopen GC, DiGirolamo C, Phinney DG. 2001. MicroSAGE analysis of 2353 expressed genes in a single cell-derived colony of undifferentiated human mesenchymal stem cells reveals mRNAs of multiple cell lineages. Stem Cells 19:408–418.

Umezawa A, Maruyama T, Segawa K, Shadduck RK, Waheed A, Hata J-I. 1992. Multipotent marrow stromal cell line is able to induce hematopoiesis in vivo. J Cell Physiol 151:197–205.

Weiss L. 1976. The hematopoietic microenvironment of bone marrow: an ultrastructural study of the stroma in rats. Anat Rec 186:161–184.