

Review Article

Two MSCs: Marrow stromal cells and mesenchymal stem cells

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Marrow stromal cells (MSC1) are able to generate a series of terminally-differentiated cells *in vitro*. Most experiments are performed with heterogeneous stromal cells obtained by adherence to plastic culture dishes. Since bone marrow-derived stromal cells are purified to a homogeneous population meeting the criteria for non-hematopoietic stem cells, these cells have been termed “mesenchymal stem cells” and have the capability of generating an array of cells. However, “mesenchymal stem cells” (MSC2) are also actual multi-purpose cells capable of differentiating into cells of mesoderm-origin regardless of cell sources. MSC2 can be recovered from a variety of other tissues, such as fat, muscle, menstrual blood, endometrium, placenta, umbilical cord, cord blood, skin, and eye. The terms “mesenchymal stem cell” and “marrow stromal cell” have been used interchangeably in emerging literature to describe cells that can be used in regenerative medicine, thereby introducing a degree of confusion. In this review, we re-organize the understanding of the two MSCs, describe their biology and differentiate between the two.

Rec./Acc.1/5/2007, pp28-36

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Key words transdifferentiation, celltherapy, epigenetics, senescence

Introduction

Two MSCs, i.e., marrow stromal cells (MSC1) and mesenchymal stem cells (MSC2), are attracting a great deal of attention, as they represent a valuable source of cells for use in regenerative medicine, as well as offering an excellent model of cell differentiation in biology. However, confusion exists in the literature due to poor application or misuse of the terms and nomenclature.

In general, mesenchymal stem cells are multi-potential stem cells that can differentiate into a variety of cell types (ref. [\[en.wikipedia.org/wiki/Mesenchymal_stem_cell\]\(http://en.wikipedia.org/wiki/Mesenchymal_stem_cell\)\). They have been shown to differentiate, *in vitro* or *in vivo*, into osteoblasts, chondrocytes, myocytes, adipocytes and neuronal cell among others. Mesenchymal stem cells have traditionally been obtained from bone marrow, and have commonly been referred to as “marrow stromal cells” \(MSC1\).](http://</p></div><div data-bbox=)

While the terms “marrow stromal cell” (or “stromal cell”) and “mesenchymal stem cell” have frequently been used interchangeably, they are increasingly recognized as separate entities as:

1. Stromal cells (MSC1) are a highly-heterogenous cell population, usually derived from bone marrow, consisting of multiple cell types with different potentials for proliferation and differentiation.

2. Mesenchymal stem cells (MSC2) encompass cells derived from other non-marrow tissues, such as fat, muscle, menstrual blood, endometrium, placenta, umbilical cord, cord blood, skin, and eye.

Bone marrow-derived mesenchymal stem cells or bone marrow stromal cells (MSC1) were discovered by Friedenstein in 1976, who described clonal, plastic-adherent cells from bone marrow that were capable of differentiating into osteoblasts, adipocytes, and chondrocytes. More recently, investigators have demonstrated that mesenchymal stem cells (MSC2) *per se* can be recovered from a variety of adult tissues and have the capacity to differentiate into a variety of specialist cell types. This review describes the recent advances in understanding of the two MSC cells, their biology and ongoing investigation and use.

Somatic stem cells

Somatic stem cells have been identified in hematopoietic⁽¹⁾, hepatic⁽²⁾, epidermal⁽³⁾, gastrointestinal⁽⁴⁾, neural^(5,6), muscle⁽⁶⁾, and bone marrow⁽⁶⁻⁸⁾ tissues. Many researchers have since demonstrated the developmental pluripotency of these cells. Bone marrow-derived stem cells can be transdifferentiated into multilineage cells, such as muscle⁽⁹⁾ of mesoderm, lung⁽¹⁰⁾ and liver^(10,11) of endoderm, and brain⁽¹²⁻¹⁵⁾ and skin⁽¹⁰⁾ of ectoderm. Somatic stem cells are more desirable than embryonic stem (ES) cells for cell therapeutics because of ethical considerations and the possible immunologic rejection of ES cells. Mesenchymal stem cells have become the most popular somatic stem cells in medicine and biology, not least because of their high reproductive capability *in vitro*.

Bone marrow stromal cells (MSC1)

The existence of non-hematopoietic cells in bone marrow was first suggested by Cohnheim about 130 years ago⁽¹⁶⁾. Bone marrow-derived stromal cells (MSC1) can differentiate into most somatic cells, including osteoblasts, chondrocytes, myoblasts, cardiomyocytes⁽¹⁷⁻²¹⁾, and adipocytes, when placed in appropriate *in vitro*⁽²⁰⁾ and *in vivo* environments⁽²²⁾, and thus are a useful cell source for regenerative medicine⁽²³⁾. Recent studies suggest that MSC1 can also differentiate into a neuronal lineage⁽²⁴⁾, and murine bone marrow-derived adult progenitor cells can differentiate into dopaminergic neuronal cells^(25,26). Since the use of MSC1 entails no ethical or immunological problems, and bone

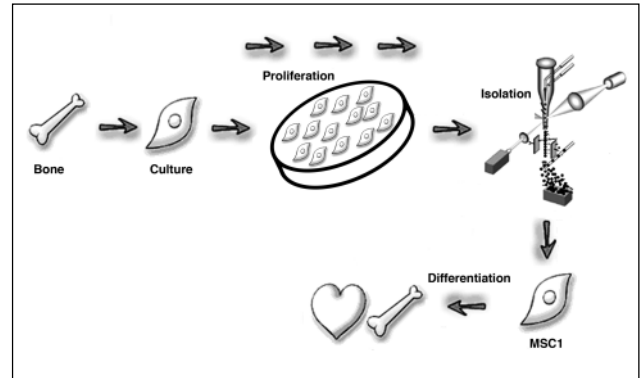


Fig.1 Development and differentiation of mesenchymal stem cells derived from bone marrow

marrow aspiration is an established routine procedure, these cells provide a useful and almost routine source of material for transplantation and tissue repair or regeneration (Fig.1).

1) Osteogenesis

KUSA-A1 cells, a murine marrow stromal cell line, are capable of generating mature bone *in vivo*⁽²⁷⁾. They are a unique, mature osteoblast cell line and serve as a very suitable model for *in vivo* osteogenesis. Bone forms in subcutaneous tissue after subcutaneous injection of the cells into mice. The osteogenesis by KUSA-A1 is not mediated by chondrogenesis and thus is considered to be membranous ossification. Follow-up study on the fate of bone by immortalized osteoblasts shows that the ectopically-generated bone keeps its size and shape for 12 months⁽²¹⁾. Furthermore, the implanted cells do not metastasize like tumor cells. These unique characteristics of KUSA-A1 cells provide an opportunity to analyze the process of membranous ossification in detail.

2) Chondrogenesis

Chondrocytes differentiate from mesenchymal cells during embryonic development⁽²⁸⁾ and the phenotype of the differentiated chondrocyte is characterized by the synthesis, deposition, and maintenance of cartilage-specific extracellular matrix molecules, including type II collagen and aggrecan⁽²⁹⁻³¹⁾. The phenotype of differentiated chondrocytes is rapidly lost since it is unstable in culture⁽³²⁻³⁵⁾. This process is referred to as “dedifferentiation” and is a major impediment to use of mass cell populations for therapy or tissue engineering of damaged cartilage. When isolated chondrocytes are cultured in a monolayer at low density, the typical round chondrocytes morphologically transform into flattened fibroblast-like cells, with profound changes in biochemical and genetic characteristics, including reduced synthesis of type II collagen and cartilage proteins⁽³⁶⁾. When cultured

three-dimensionally in a scaffold such as agarose, collagen, and alginate, redifferentiated chondrocytes re-express the chondrocytic differentiation phenotype.

KUM5 mesenchymal cells, a MSC1 line, generate hyaline cartilage *in vivo* and exhibit endochondral ossification at a later stage after implantation³⁷). OP9 cells, another MSC1 line, derived from macrophage colony-stimulating factor-deficient osteopetrotic mice, and also known to be niche-constituting cells for hematopoietic stem cells, express chondrocyte-specific or -associated genes, such as type II collagen β 1, Sox9, and cartilage oligomeric matrix protein at an extremely high level, as do KUM5 cells. OP9 micromasses exposed to TGF- β 3 and BMP2 form type II collagen-positive hyaline cartilage within two weeks *in vivo*. The unique characteristics of KUM5 and OP9 cells provide an opportunity to analyze the process of endochondral ossification.

3) Cardiomyogenesis

It has been generally accepted that cardiac myocytes are unable to divide once cell proliferation ceases shortly after birth in the mammalian heart, because mitotic figures have not been detected in myocytes³⁸). Cardiomyocytes induce DNA synthesis *in vivo* and *in vitro*^{39,40}). Adult hearts often exhibit a polypoid structure, which results from stochastic accumulation of mutations as cells pass through cell-cycle checkpoints⁴¹). Bone marrow-derived stromal cells (MSC1) are able to differentiate into cardiomyocytes *in vitro* and *in vivo*^{19,20,42,43}) and a hierarchical model has been proposed for this *in vitro* cardiomyogenic differentiation. MSC1 in culture include a mixture of at least three types of cells, i.e., cardiac myoblasts, cardiac progenitors and multi-potential stem cells, and a follow-up study of individual cells suggests that commitment of a single-cell-derived stem cell toward a cardiac lineage is stochastic⁴⁴). Furthermore, MSC1 over-expressing well-known master transcription factors, i.e., Csx/Nkx2.5 and GATA4, unavoidably undergo cardiomyogenic fate and behave like transient amplifying cells. MSC1 also transdifferentiate into cardiomyocytes in response to humoral factors, such as demethylation of the genome, in addition to environmental factors (See the chapter “Epigenetic modifier as a differentiating inducer”).

4) Neurogenesis

MSC1 can exhibit neural differentiation when exposed to demethylating agents¹⁴): the cells differentiating into three types of neural cells, i.e., neurons, astrocytes, and oligodendrocytes. With exposure to basic fibroblast growth factor, nerve growth factor, and brain-derived neurotrophic factor, the transdifferentiation of human stromal cells is limited to neurons¹⁴). The change

in gene expression during differentiation is global and drastic⁴⁵): the differentiated cells no longer exhibit the profile of stromal cells or the biphenotypic pattern of neuronal and stromal cells. Osteoblasts capable of intra-membranous ossification are likely to differentiate into neuronal lineages, but adipocytes do not¹⁴). Interestingly, the cranio-facial membranous bones develop from the neural crest, which is of ectodermal origin. Development naturally progresses from neural crest cells to terminally-differentiated osteoblasts⁴⁶). The finding of *in vitro* differentiation from mesoderm- to ectoderm-derived cells is thus the opposite of the developmental process, i.e., from ectoderm- to mesoderm-derived cells. Converting differentiated osteoblasts or MSC1 to neuronal cells, a key future task for any cell-based therapy, would thus oppose the usual direction of cell differentiation. This can now be achieved by exposing stromal cells to neurotrophic factors, at least *in vitro*.

Dopaminergic neuron-associated genes, such as *nurr1* and *wnt-5a*, are induced at an extremely high level in the neuronally-differentiated stromal cells. *Wnt5a* and *nurr1* are involved in the differentiation of mid-brain precursors into dopaminergic neurons^{25,26}). It is quite significant that dopaminergic neurons can be generated from MSC1, since they are one of the key targets for regenerative medicine.

Epigenetic modifier as a differentiating inducer

The demethylating agent, 5-azacytidine, is a cytosine analog that has a remarkable effect on transdifferentiation of cells and has been shown to induce differentiation of stromal cells into cardiomyocytes, skeletal myocytes, adipocytes, and chondrocytes^{19,42,47}). The effect of this low-molecular substance is not surprising, since it is incorporated into DNA and has been shown to cause extensive demethylation. The demethylation is attributable to covalent binding of DNA methyltransferase to 5-azacytidine in the DNA⁴⁸), with subsequent reduction of enzyme activity in cells resulting in dilution-out and random loss of methylation at many sites in the genome. This may, in turn, account for the reactivation of cardiomyogenic “master” genes, such as MEF-2C, GATA4, dHAND, and Csx/Nkx2.5, leading to stochastic transdifferentiation of MSC1 into cardiomyocytes. Use of 5-azacytidine is beneficial, but since it may have drawbacks, i.e., gene activation leading to oncogenesis and undesired differentiation, care must be exercised before using it to induce cells to differentiate into target phenotypes. Immortalized cells, including marrow stromal cells, have specific patterns of DNA methylation. The established methylation pattern of cells is maintained

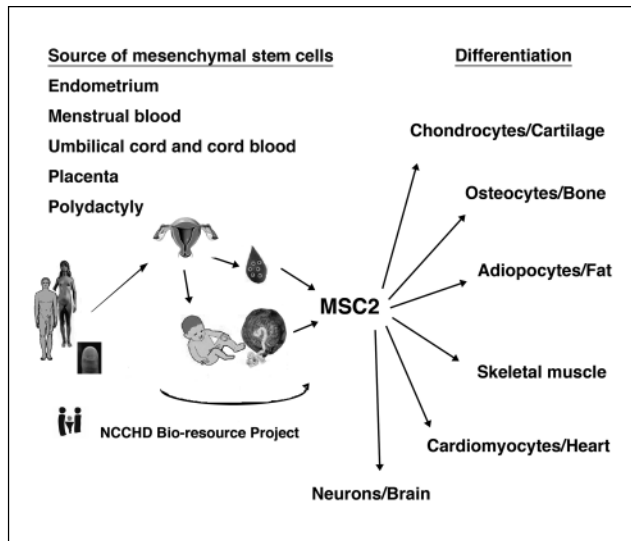


Fig.2 Sources and differentiation of mesenchymal stem cells

with considerable fidelity and silenced genes are stably inherited throughout the culture period⁴⁹⁻⁵¹). The demethylating agent induces differentiation by altering the original methylated pattern and reactivating the silenced genes.

Mesenchymal stem cells (MSC2)

Tissues originating in the mesoderm include blood cells, blood vessels, heart, bone, cartilage, fat, skeletal muscle, tendon, and tissue mesenchyme. Blood cells in bone marrow are the elements that create the concept of stem cells, but bone marrow includes another cell group, i.e., mesenchymal stem cells (MSC2), which possess adherent properties. These cells have the ability to differentiate into a variety of cells and may have an organ maintenance mechanism that serves as back-up. Human mesenchymal stem cells (MSC2) are a useful source of cells for transplantation for several reasons: they have the ability to proliferate and differentiate into mesodermal tissues and they entail no ethical or immunological problems. MSC2 have been studied extensively over the past three decades and numerous independent research groups have successfully isolated them from a variety of sources, most commonly from bone marrow^{19,22,52-55}). Yet, in addition to bone marrow, almost all human tissues or organs can be a source of mesenchymal stem cells, since they all have stroma or mesenchyme as well as parenchyma or epithelium.

Available mesenchymal cell lines and mesenchymal cells in culture

MSC2 have been extracted from fat, muscle, menstrual blood,

endometrium, placenta, umbilical cord, cord blood, skin, and eye (Fig.2). Moreover, the source tissues can be obtained without difficulty from resected tissues at surgery and from birth deliveries (<http://www.nch.go.jp/reproduction/cellbank2.htm> and <http://www.nch.go.jp/reproduction/cells/primary.html>); menstrual blood can be provided from volunteers. The placenta is composed of amniotic membrane, chorionic villi and decidua, each of which can be a source of different types of MSC2. Large numbers of MSC2 can be easily obtained because the placenta is usually provided for research purposes. Menstrual blood also contains a large number of MSC2, although it is usually regarded as waste material.

We have also isolated many specific cell lines from adhering cells of mouse bone marrow (<http://www.nch.go.jp/reproduction/cellbank2.htm>) as follows:

- Multi-potential stem cell line: 9-15c cells (originally KUM2 cells) have multi-potential allowing differentiation into bone, fat, skeletal muscle, and myocardial cells through continued passage;
- Oligo-potential cell lines: KUM9 cells that lose the ability to differentiate to myocardial cells but retain differentiation to bone, fat, and skeletal muscle and NRG cells that lose the capability to differentiate into myocardial cells and skeletal myocytes but retain differentiation to bone and fat;
- Bi-potential cells: KUSA-O cells are capable of differentiating into osteoblasts and adipocytes;
- Precursor cells: KUSA-A1 and H-1/A are osteoblasts and preadipocytes, respectively. Adipogenic 3T3-L1⁵⁶), osteogenic MC3T3-E1⁵⁷), and chondrogenic ATDC5 cells⁵⁸) have been isolated from stem cells of a mesenchymal nature.

Focusing on human MSC2 derived from umbilical cord blood (UCBMSC) as an example, isolation, characterization, and differentiation of clonally-expanded UCBMSCs have been reported^{59,60}), and UCBMSCs have been found to have multi-potential⁶¹). Most of the surface markers are the same as those detected in their bone marrow counterparts⁴²), with both UCB- and bone marrow-derived cells being positive for CD29, CD44, CD55, and CD59, and negative for CD34 and CD117. Significantly, the differentiation capacity of UCB-derived cells is unaffected during establishment of a plate-adhering population of cells from UCB.

Life span of MSC1 and MSC2

Marrow stromal cells (MSC1) and mesenchymal stem cells (MSC2) are useful for cell transplantation. However, it is difficult to study and apply them because of their limited life span.

One of the reasons for this is that normal human cells undergo a limited number of cell divisions in culture and then enter a non-dividing state called “senescence”^{62,63}. Human cells reach senescence after a limited number of cell replications, and the average number of population doublings (PDs) of marrow-derived mesenchymal stem cells has been found to be about 40⁴², implying that it would be difficult to obtain enough cells to restore the function of a failing human organ. Large numbers of cells must be injected into damaged tissues to restore function in humans, and cells sometimes need to be injected throughout entire organs.

A system that allows human cells to escape senescence by using cell-cycle-associated molecules may be used to obtain sources of material for cell therapy^{64,65}. Both inactivation of the Rb/p16INK4a pathway and activation of telomerase are required for immortalization of human epithelial cells, such as mammary epithelial cells and skin keratinocytes. Human papillomavirus E7 can inactivate pRb, and Bmi-1 can repress p16INK4a expression. Inactivation of the p53 pathway is also beneficial, even if not essential, to extension of the life span⁶⁶. Human marrow stromal cell strains with an extended life span can be generated by transduction of combination of TERT, and Bmi-1, E6 or E7⁴⁵. Cells with extended life span grow *in vitro* for over 80 PDs, and their differentiation potential is maintained. Transfection of TERT alone is insufficient to prolong the life span of marrow stromal cells, despite TERT having been reported to extend the life span of cells beyond senescence without affecting their differentiation ability⁶⁷. Human stromal cells transfected with TERT and Bmi-1, E6 or E7 do not transform according to the classical pattern: they do not generate tumors in immunosuppressed mice; they do not form foci *in vitro*; and they stop dividing after confluence. The possibility that gene-transduced stromal cells might become tumorigenic in patients several decades after cell therapy therefore cannot be ruled out. Nevertheless, these gene-modified stromal cells may be used to supply defective enzymes to patients with genetic metabolic diseases, such as neuro-Gaucher disease, Fabry disease, and mucopolysaccharidosis, which have a poor prognosis and are sometimes lethal. The “risk versus benefit” balance is essential when applying these gene-modified cells clinically, and the “risk” or “drawback” in this case is transformation of implanted cells. These marrow stromal cells (MSC1) with prolonged life span also provide a novel model for further study of cancer and stem cell biology.

Differentiation of mesenchymal stem cells

Retroviral labeling of individual cells is a useful clonal assay

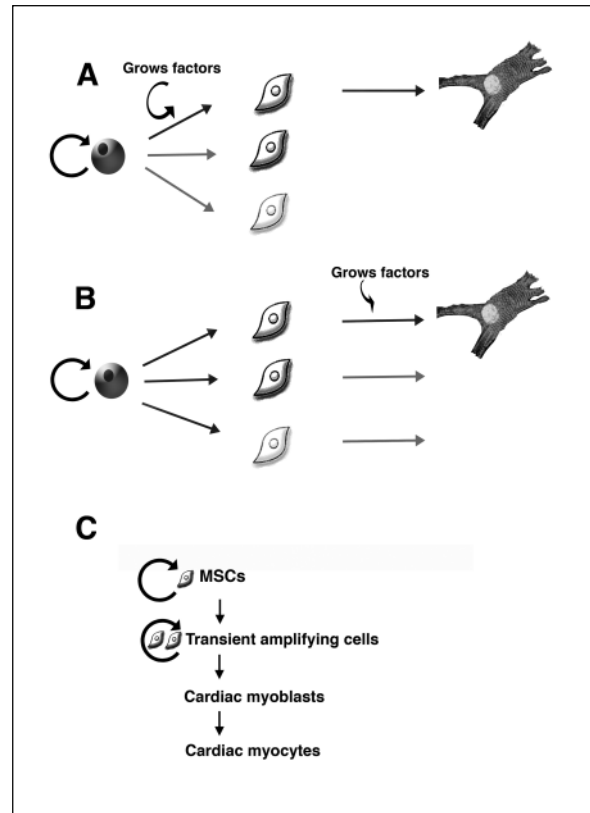


Fig.3 Model of stem cell differentiation

A. Deterministic model.

B. Stochastic model.

C. Differentiation model of mesenchymal stem cells.

to monitor lineage commitment at the single cell level. At present, several models have been proposed in which hematopoietic lineage determination is driven intrinsically⁶⁸, extrinsically⁶⁹, or both⁷⁰. The issue of the mechanism and the extent of cellular differentiation that occurs when stem cells begin to differentiate is the area of furthest advanced research. Two models have been proposed: a deterministic model, in which differentiation is governed by the microenvironment (including growth factors and cytokines), and a stochastic model, in which differentiation, self-replication and the direction of differentiation emerge somewhat randomly (Fig.3A,B). The different models arise from different conceptions of mesenchymal stem cells. The mesenchymal stem cell (MSC2) line is stochastically committed toward the cardiac lineage, and following this commitment, they proliferate as transient amplifying cells and differentiate into cardiac myocytes (Fig.3C).

Considering stem cell transplant as a therapy, when mature cells arising from hematopoietic stem cells are needed, as in marrow transplant, there are no problems attending cellular dif-

ferentiation. However, in the case of cells that serve to originate cells of several different organs, as in the case of mesenchymal stem cells, there is a possibility for differentiation to cells not needed in the treatment. Ectopic tissue may therefore emerge from implanted mesenchymal stem cells, especially where the buffering system from a given site is lost and the stem cells begin to differentiate randomly into cells differing from the implanted site, thereby creating unwanted ectopic tissue.

Conclusion

Mesenchymal stem cells can be isolated from bone marrow by standardized techniques and expanded in culture through many generations, while retaining their capacity to differentiate along set pathways when exposed to appropriate conditions. This property opens up therapeutic opportunities for the treatment of lesions in mesenchymal tissues, and protocols have been devised for the treatment of defects in articular cartilage⁷¹⁾, bone⁷²⁾, tendon⁷³⁾, and meniscus⁷⁴⁾ and for bone marrow stromal recovery⁷⁵⁾ and osteogenesis imperfecta⁷⁶⁾.

In this context, we prefer to use the word “stroma” rather than “mesenchymal stem cells” for accuracy and to avoid confusion. In the field of hematopoiesis, marrow stroma were originally treated as “second class citizens”⁷⁷⁾, and represented a niche field. Today, marrow stroma are a “major player” in regenerative medicine and stem cell biology and are no longer viewed as a peripheral field of research. In addition, there is also a rapidly growing body of research into the biology and potential use of true “mesenchymal stem cells” derived from other human tissues, which are showing significant promise for future therapy, reparation or regeneration of human tissues and organs.

Clearly, this field is in its relative infancy, our understanding is at present limited but the potential benefits are great. We should perhaps, therefore, remember that the unexpected and unrivalled potential of MSCs to differentiate into a wide variety of cells represents a gift not a privilege and, with respect to the two MSCs, we should recognise and welcome their role in medicine with the words “with great power comes great responsibility”.

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