Regenerative medicine for bone diseases using mesenchymal stem cells

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Since the monumental publication in 1998 by Pittenger et al., mesenchymal stem cells (MSCs) have been a center player of regenerative medicine and now a number of clinical trials using MSCs have been conducted in various fields of tissue regeneration including those of bone. It cannot be denied that due to enthusiastic clinical demanding, clinical application of MSCs has launched with little knowledge concerning the nature of native MSCs. Recent advances, however, have gradually revealed enigmatic biological properties of MSCs, which subsequently requires the reconsideration of minimum criteria of this type of stem cells. Plastic adherence was no more an absolute requirement of MSCs, and there seemed to be CD34⁺ MSCs. In addition, in vitro multidirectional property does not guarantee such property in vivo. As a more fundamental issue, cell-of-origin of MSC may be not single, and there seemed to be at least ectodermal (neural crest) MSCs and mesoderm (perivascular) MSCs. Accumulation of preclinical and clinical data has also revealed the role of MSCs in bone regeneration. Against to the initial expectation, the role of MSCs as cell sources to participate bone regeneration seemed to be less significant than those as producer of materials to induce bone regeneration by host cells. The later role may open a new venue of regenerative medicine, which may be called cell-free cell therapy. Understanding of these important features and function of MSC will greatly improve the value of MSCs and promote the proper application of these cells in bone repair and regeneration.

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Introduction

The multipotent precursors of the bone marrow stroma were the first adult stem cells to be identified and are referred as mesenchymal stem cells (MSC)¹). In the bone marrow, MSCs represent about the 0.01% of the mono-nuclear cells and provide the structural and functional sup-
Cell-of-origin of MSC

Because the mesenchyme derives mainly from mesoderm and ectoderm, it is reasonable to consider cell-of-
origin in these two germ layers. As for ectodermal origin, Takashima et al. reported that the earliest wave of MSC is generated from Sox1+ neuroepithelium but not from mesoderm, and that Sox1+ neuroepithelium gives rise to MSCs in part through a neural crest intermediate stage\(^\text{10}\). MSC recruitment from this pathway, however, is transient and is replaced by MSCs from unknown sources. Morikawa et al. demonstrated that MSCs formed spheres that expressed neural crest stem cell genes labeled by GFP and differentiated into neurons, glial cells, and myofibroblasts\(^\text{11}\). Interestingly, MSCs were found both in the GFP\(^+\) and GFP\(^-\) fraction and there were no significant differences in the in vitro characteristics between these two populations, suggesting that MSCs in adult bone marrow have at least two developmental origins, one of which is the neural crest\(^\text{11}\). As for mesodermal origin, perivascular cells have been in attention. Crisan et al. showed that long-term cultured perivascular cells retained multidirectional differentiation property including myogenic, osteogenic, chondrogenic, and adipogenic potentials, and expressed MSC markers. They also showed that expression of MSC markers was also detected at the surface of native, non-cultured perivascular cells, indicating the blood vessel walls harbor a reserve of progenitor cells that may be the origin of MSCs\(^\text{12}\). These two origins may not be mutually exclusive. In the developmental study of mice, there is a bi-lineage stem cell (axial stem cell), the fate of which was determined by single transcription factor (Tbx6)\(^\text{13}\).

**Homing as an important feature of MSCs**

An important distinguishing feature of MSCs compared to most other cell-type is that MSCs retain the ability to migrate to differentiated tissues. A number of studies have clearly demonstrated that when MSCs are systemically or locally administered, they selectively home to sites of injury\(^\text{14}\). Why MSCs specifically home to these sites and what damaged tissues have that attract MSCs are still open questions, but inflammation is most likely the responsible denominator. Among the chemotactic chemokines involved in MSC homing, stromal cell-derived factor 1 seems to function as a reservoir. Recently, bone marrow cells expressing CXCR4 (CAR cells) can differentiate into osteoblasts and adipocytes, suggesting the function as MSCs\(^\text{15}\).

**MSC as a factory to fabricate carpenters**

Most of initial cell transplantation studies were designed and performed aiming that transplanted cells were engrafted to regenerate the tissue. Recent experimental studies, however, showed it was not the case. Only a small proportion of MSCs, locally or systemically administrated, will actually be incorporated into injured tissues, indicating that the beneficial effects in tissue repair and regeneration is more likely indirect and depends on the paracrine activity of MSCs. To understand the mechanisms of paracrine effects, several comprehensive analyses of soluble factors has been done, but it seems difficult to explain the pleiotrophic effects of MSC by cytokines and growth factors alone. These facts have raised the attention to exosome produced by MSC\(^\text{16}\). Exosome is a vesicle with nano-size that is derived from intracellular components known as multi-vesicular bodies (MVs), which contain proteins, mRNA, or miRNA. Therefore exosome has remarkable features including the ability to transfer not only proteins but also functional genetic materials such as RNA to other cells, which may modify the expression profile of recipient cells. Kim et al. characterized 730 proteins in MSC-derived MVs, and found that a number of cell surface markers such as PDGFR, EGFR, signaling molecules such as RAS-MAPK pathway, cell adhesion molecules that support possible role of such vesicles in tissue repair\(^\text{17}\). As for genetic materials, Collino et al. performed comparative miRNA profiling between those of MV and original cells and found that some miRNAs appeared to have been selectively sorted into MVBs as there were not detectable in the cells\(^\text{18}\).

**MSCs for bone regeneration**

Fridenstein was the first to show that new bone was formed by proliferative fibroblast-like marrow cells\(^\text{19}\). Based on this pioneering study, orthopedic surgeons have been implanting bone marrow to look for their effect for bone repair and regeneration in various clinical settings without scientific rationale. It is after mid 1990 that prospective clinical trials started, and now the effect of implantation was scientifically confirmed. Therefore, although it started far before the concept of MSC was proposed, the implantation of bone marrow for bone condition may be regarded as the first clinical application of MSC.

**Application to facture repair**

Bone has an ability to regenerate and the healing of fracture is usually considered to be biologically easy, but in a
few cases fracture sites fail to unite or the process delays remarkably, which are called nonunion or delayed union, respectively. Implantation of bone marrow aspirate to fractures sites, with or without the process of concentration, has been used to accelerate the healing process for such condition and successful results were reported\(^{20}\). The fate of implanted bone marrow cells, however, has not yet been shown. In the fracture healing model of mice, implanted MSCs were accumulated in fracture sites by CXCR4-dependent manner and contributed callus formation by expressing BMP2\(^{27}\). Bone marrow implantation was also applied to congenital pseudoarthrosis of tibia (CPT), which is a rare orthopedic disease presenting spontaneous fractures that do not heal and usually associated with neurofibromatosis type I. Granchi et al. reported that the bony union was obtained in 3 out of 10 cases of refractory CPT, and that \textit{in vitro} mineralization activity of MSC corresponded with clinical outcome\(^{22}\).

**Application to osteonecrosis**

Osteonecrosis is a progressive degenerative disease that results from interruption of blood supply to the bone and subsequent loss of bone forming cells. This condition can occur in any bone, but most frequent sites is femoral head (osteonecrosis of femoral head, ONF). Core decompression is the classical way to treat ONF patients at early stage, and the combination of this method with autologous bone marrow implantation has initiated at 1990\(^{20}\), and long-term follow-up studies confirmed the effect of implantation\(^{24}\). Application of \textit{in vitro} expanded MSCs to ONF were also performed. Zhao et al. performed a randomized trial of core decompression with or without cell transplantation, and reported that the patients in the later group showed significantly better clinical and radiological results\(^{25}\). Although these data are promising, the application of this procedure was limited to early stage (I or II) of ONF, and patient with stage III showed poor results\(^{23, 24}\). Based on the result of animal study\(^{26}\), we have started the clinical trial using in vitro expanded MSC with vascularized fibular bone graft for patients with late stage.

**Osteogenesis imperfect**

Osteogenesis imperfecta (OI) is a hereditary condition with a defect of type I collagen gene. Due to the mutant amino acid impedes the structure of triple helix, bone tissue turn to be extremely fragile. Transplantation of whole bone marrow as well as \textit{ex vivo}-expanded MSCs leads to clinical benefits in children with OI, such as the increase of total mineral bone content and reduction of fracture frequency, suggesting the contribution of donor derived MSCs\(^{27}\). From the results of mice study, however, non-(plastic)-adherent bone marrow cells (NABMCs) are more potent osteoprogenitors than MSCs in mice. The donor NABMCs differentiate to osteoblasts, they contribute normal collagen to the bone matrix. In contrast, MSCs do not substantially engraft in bone\(^{28}\).

**Conclusions**

In most cases, MSCs used in current clinical trials are actually a heterogenous cell mixture of mesenchymal stromal cells, the abbreviation of which is also MSC. These two “MSC” have been used without careful discrimination.
Recent progress in “mesenchymal stem cell” biology, however, clearly indicated that the current definition of and concept of therapeutic effect of “mesenchymal stem cell” should be revisited. Now it is the time to use two MSCs separately, which will accelerate our understanding of MSCs and improve access to well-designed clinical trials.

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