Special Issue: Mesenchymal Stem Cells

Mini Review

Regenerative medicine for bone diseases using mesenchymal stem cells

Junya Toguchida[1, 2, 3)]

[1)]Department of Tissue Regeneration, Institute for Frontier Medicine, [2)]Depatment of Growth and Differentiation, Center for iPS Cell Research and Application, [3)]Department of Orthopaedic Surgery, Kyoto University, Kyoto, Japan

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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*Correspondence should be addressed to:
Junya Toguchida, M.D., Ph.D. Professor, Department of Tissue Regeneration, Institute for Frontier Medical Sciences, Kyoto University, 53 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan. Phone: +81-75-751-4134, Fax: +81-75-751-4646, E-mail: togjun@frontier.kyoto-u.ac.jp

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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 mononuclear cells and provide the structural and functional sup-

port for hematopoietic stem cells (HSCs) in their niche[2]. However, MSCs localize in various mesenchymal tissues other than bone marrow such as placenta, umbilical cord blood, adipose tissue, skeletal muscle, peripheral blood cells, and synovium[3]. Trials to apply MSCs for various field of regenerative medicine has launched also in Japan. The Ministry of Health, Labor and Welfare enacted the official guideline to use adult stem cells for clinical trials in 2006, and since then 36 clinical trials using stem cells were approved until the end of 2011. Among those trials, MSCs were used as cell sources in more than two thirds of cases, in which MSC derived from bone marrow were used in most of cases, but those from adipose tissues, and umbilical cord was used as well. There are a couple of backgrounds to explain why MSCs are so widely used in this field. First, because MSCs can differentiate into various types of mesenchymal cells, they are suitable cell sources for cell therapy to regenerate lost mesenchymal tissues. Bone regeneration by MSC-derived bone-producing cells is a typical example of this type of application. Accessibility is another important factor, and in this meaning adipose tissue is gaining more and more interest among the various sources, because adipose-derived MSCs are available in large amounts from liposuction procedures. Also, MSC can serve as cytokine-producers, which will create a suitable environment for tissue regeneration by host cells including enhancement of angiogenesis or inhibition of inflammatory reaction. Homing is another unique feature of MSCs, which facilitates the repair of damaged tissues. In spite of these promising features for clinical application, the basic biology of MSCs has been an issue-to-be-analyzed for long time. Recent advance in this aspect, however, revealed several important biological features of MSC. At the same time, the functional roles of MSCs in tissue regeneration also gradually have been discovered. Against to the initial expectation, the contribution of MSCs as cell source for tissue regeneration was much less than as cytokine-producing cells. This mini review summarizes recent findings pertaining to the definition and characterization of MSCs and highlights novel mechanisms of their actions in regenerating of bone *in vivo*.

Reconsideration of definition

The minimal requirements as MSCs defined by the International Society for Cellular Therapy are; 1) plastic adherence, 2) expression of CD73, CD90 and CD105, and negativity for various hematopoietic markers, and 3) ability to differentiate into mesenchymal cell types including adipocytes, chondrocytes, and osteoblasts[3]. Researchers have been using these criteria to prove the authenticity of their MSCs, although recent studies have raised serious concern about these criteria. First, plastic adherence does not appear an essential characteristic of MSCs. Recent studies from multiple laboratories have shown the existence of non-adherent MSC subpopulations that display the same multidirectional potential of adherent MSCs[4]. Moreover, the non-adhernet MSCs present the same ability to migrate to damaged tissues *in vivo* as adherent MSC and also function in tissue repair and regeneration[4]. The surface antigen pattern is also an issue to be reconsidered. A number of studies have been shown the presence of $CD34^+$ cells with MSC-like properties[5]. These cells were non- or low adherent cells, indicating that non-adherent MSC-like cells retain the feature of common precursors of mesenchymal and hematopoietic lineages. The expression of such hematopoietic markers may disappear under the adherent culture condition, and thus the significance of cell surface antigen expression should be carefully evaluated because the expression of each antigen may fluctuate considerably by culture condition[3]. As for the differentiation property, the difference between *in vitro* and *in vivo* is coming an issue. Using a pulse-labeled system, Park et al. demonstrated that $Mx1^+$ cells, which showed multidirectional differentiation property *in vitro*, had only osteo-lineage restricted property *in vivo* in growing and adult mouse[6], indicating that bone-marrow-derived MSCs may be a heterogeneous population with the $Mx1^+$ population, representing a highly dynamic and stress responsive stem cell population of fate-restricted potential. This finding may have some relevance with previous reports demonstrating the existence of a "super MSCs" in subpopulation within bone marrow-derived MSCs[7-9]. These multipotent cells were capable of differentiating not only into mesodermal-lineage, but also into other lineages of the ectodermal and endodermal germ layers. Because most of these cells were initially identified under severe condition such as serum-free culture, the multipotency of these cells might be induced *in vitro*.

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[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[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[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[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)[13].

Homing as a 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[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[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[16]. Exsosome is a vesicle with nano-size that is derived from intracellular components known as multi-vesicular bodies (MVBs), 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[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[18].

MSCs for bone regeneration

Fridenstein was the first to show that new bone was formed by proliferative fibroblast-like marrow cells[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

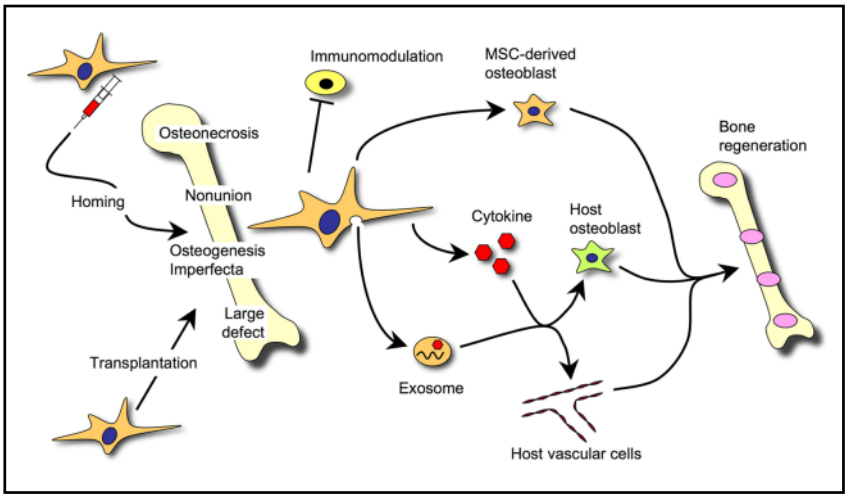

Fig.1 The role of MSCs in bone repair and regeneration
MSC-derived osteoblasts, host-derived osteoblasts, and host-derived other cells such as vasculo-endothelial cells coordinate the tissue regeneration.

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[21]. 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 *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[23], and long-term follow-up studies confirmed the effect of implantation[24]. Application of *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 impercta (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 *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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Disclosure of conflict of interests

No conflict of interests

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