

REVIEW ARTICLE

Circulating mesenchymal stem cells and their clinical implications



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Summary Circulating mesenchymal stem cells (MSCs) is a new cell source for tissue regeneration and tissue engineering. The characteristics of circulating MSCs are similar to those of bone marrow-derived MSCs (BM-MSCs), but they exist at a very low level in healthy individuals. It has been demonstrated that MSCs are able to migrate to the sites of injury and that they have some distinct genetic profiles compared to BM-MSCs. The current review summarizes the basic knowledge of circulating MSCs and their potential clinical applications, such as mobilizing the BM-MSCs into circulation for therapy. The application of MSCs to cure a broad spectrum of diseases is promising, such as spinal cord injury, cardiovascular repair, bone and cartilage repair. The current review also discusses the issues of using of allogeneic MSCs for clinical therapy.

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Introduction

Mesenchymal stem cells (MSCs) are non-haematopoietic cells which can be easily isolated from bone marrow and other tissues, such as adipose, umbilical cord, and peripheral blood. The MSCs have a multipotent capacity to

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differentiate into a variety of other cell types, including osteoblasts, adipocytes, chondrocytes, myoblasts, and neurons [1,2]. In response to stimuli, MSCs have the ability of homing to the target tissue. Also, MSCs have been shown to be immunosuppressive and anti-inflammatory; they do not express major histocompatibility complex-II (MHC-II), CD80, CD86, and CD40, and minimally express MHC-I on the cell surface [1,3]. These characteristics have made MSCs a promising cell source for tissue engineering.

Circulating MSCs, also called peripheral blood-derived MSCs (PB-MSCs), were initially discovered as fibroblast-like cells and were later confirmed by many investigators as peripheral blood-borne colony-forming units [4,5]. Usually, they exist at a very low level in healthy individuals, but under some pathological conditions, the number of circulating MSCs is greatly increased [6,7]. In 2007, we reviewed some studies/knowledge about circulating MSCs and their relationship with bone marrow-derived MSCs (BM-MSCs) [8]. In this current review, we give a brief summary of the current studies about circulating MSCs and analyse the clinical application of circulating MSCs in tissue regeneration.

Biological characteristics of circulating MSCs

As there are no consistent defining characteristics of MSCs among researchers, the International Society for Cellular Therapy has proposed three criteria that have been generally accepted to categorise progenitor cells as MSCs: (1) adherence to plastic; (2) specific surface antigen expression; and (3) multipotent differentiation potential. Circulating MSCs also fulfil these requirements.

The frequency of BM-MSCs in humans under normal conditions is very low, ranging from 1 in 10^4 to 1 in 10^5 bone marrow mononuclear cells (MNCs) [9]. Compared with BM-MSCs, the frequency of circulating MSCs in humans is even lower, in the order of 1 in 10^8 peripheral blood MNCs [10]. Fernandez and co-workers [11] successfully identified cells with features of MSCs in growth-factor-mobilized peripheral blood cells from breast cancer patients. The MSCs identified by Fernandez and co-workers [11] expressed collagen I, collagen III, fibronectin,

CD106, CD54, SH2, and SH3, but did not express antigens CD34, CD45, and CD14. However, they did not find any stromal cells in normal peripheral blood cells which were not mobilized by growth factors. Three years later, Zvaifler and co-workers [12] successfully identified mesenchymal precursor cells in the blood of normal individuals, and these cells were referred to as blood-derived mesenchymal precursor cells. These blood-derived mesenchymal precursor cells also did not express CD34, CD45, and CD14, but were positive for vimentin, collagen I, bone morphogenetic protein receptors IA (BMPR IA) and BMPR IB. In the stromal cells obtained from mobilized peripheral blood cells, two cell populations were observed; fibroblast-like cells and some small round cells. Zvaifler and co-workers [12] also observed that both fibroblast-like cells and some large round cells exist in the predominant cells, and they found that culture conditions are an important factor which can modify the morphology of the progenitor cells.

Despite the difficulty in detection, to date, circulating MSCs have been detected and isolated from various species, such as guinea pig, mouse, rabbit, rat, and humans [8]. We have successfully isolated and cultured PB-MSCs from adult Sprague Dawley rats under normal conditions. The BM-MSCs and PB-MSCs showed similar characteristics of cell proliferation and multi-differentiation potentials. We compared the expression of a set of surface markers, and found that CD73 may be an important indicator to distinguish PB-MSCs from BM-MSCs (as summarized in Fig. 1). PB-MSCs are also plastic-adherent and have multi-differentiation potential. They can be differentiated into adipocytes, chondrocytes, and osteocytes under certain conditions, as proved by previous publications and our unpublished study.

MSCs homing

The capacity of MSCs to home and migrate to the target tissue is an important determinant for the clinical use of MSCs. For example, for bone formation to occur, MSCs must migrate to the bone surface, where they differentiate into osteoblasts and deposit bone matrix. There is a prevailing view that circulating MSCs engraft more rapidly than BM-MSCs [13]. Although the mechanisms for MSCs homing/

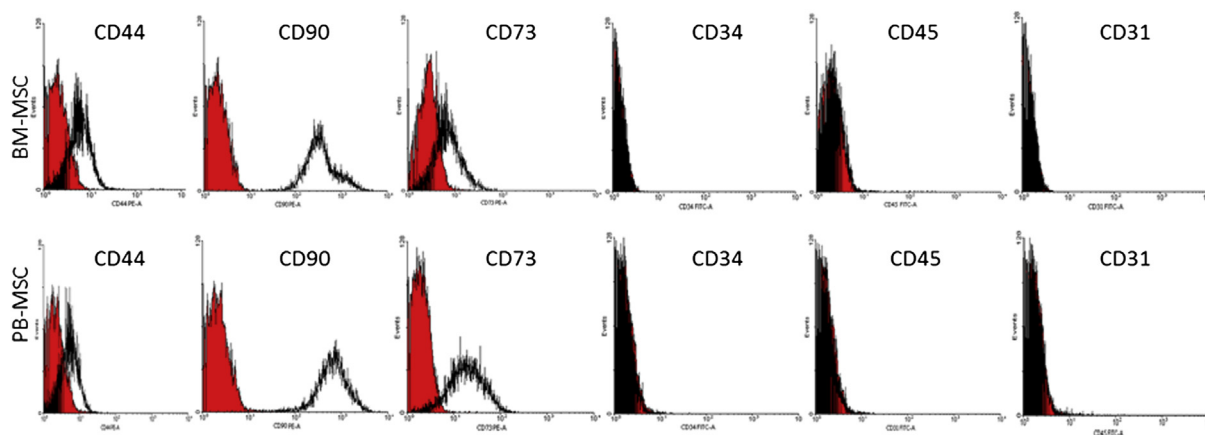


Figure 1 Surface markers of rat peripheral blood-derived mesenchymal stem cells (PB-MSCs) and bone marrow-derived MSCs (BM-MSCs), as analysed by flow cytometry. Isotype controls are represented in red, and the analysis with antibodies for cellular surface markers are represented in black. The PB-MSCs and BM-MSCs shared similar phenotypic CD markers.

migration and the mediators and chemotactic signalling involved are not fully identified, a likely paradigm for MSCs migration has already come into being. It is proposed that the mechanism used for MSCs homing is similar to the well-documented trafficking and homing of haematopoietic stem cells to the bone marrow, and leukocytes into the inflamed tissues [14,15]. Of course, there are some differences among them. For example, the MSCs do not express ligands to endothelial selectins, such as P-selectin glycoprotein ligand-1 (PSGL-1) or sialyl Lewis X carbohydrates, so MSCs are not able to bind to chimeric constructs of the endothelial selectins CD62E (E-selectin) and CD62P (P-selectin).

Recently, the chemokine stromal cell-derived factor-1 (SDF1)/CXCR4 axis has been recognized as controlling the migration of MSCs [16,17]. CXCR4, a 352 amino acid rhodopsin-like G-protein-coupled receptor, binds with SDF1 and induces intracellular signalling transduction through several divergent pathways which are related to chemotaxis, cell survival, proliferation, and gene transcription. Our previous study also showed that CXCR4 was involved in MSCs homing and engraftment to tumours, the migration potential of MSCs toward tumour cells was enhanced with the upregulation of CXCR4 when MSCs were exposed to tumour conditioned medium, and the SDF1 inhibitor, AMD3100, could partly abolish the MSCs migration toward tumour cells [18]. A big issue concerning the SDF1/CXCR4 axis is that CXCR4 is usually absent on the surface of MSCs culture-expanded *in vitro* [16,19,20]. To overcome this problem, stimulating the expression of CXCR4 is one of the strategies for enhancing the migration capacity of MSCs [21–23].

MSCs for tissue regeneration

The capacity of MSCs is outstanding, such as multi-differentiation, immunosuppression, and immune privilege. In addition, they do not have the ethical issues like embryonic stem cells. These unique privileges make MSCs a promising cell source for cytotherapy. There is accumulating evidence that MSCs can be used to cure a broad spectrum of diseases, such as spinal cord injury, cardiovascular repair, bone and cartilage repair.

For the use of MSCs in cardiovascular repair, it has been demonstrated that systemically injected MSCs migrate to the infarcted myocardium at various time points, and some of the MSCs undergo differentiation to a cardiac phenotype, whereas other cells participate in angiogenesis in the infarcted area [24]. MSCs can survive very well in a xenogeneic environment while retaining their abilities. In another study involving the autologous delivery of bone marrow cells into the infarct border zone in patients who had a myocardial infarction, all patients were alive and well at 3–9 months after surgery, and 80% (5/6) of the patients showed greatly improved infarct tissue perfusion; 70% (4/6) of the patients showed enhanced global left-ventricular function [25].

Also, there are many examples of the application of MSCs in the area of bone and cartilage repair. Quarto et al. [26] reported that local delivery of bone marrow stem cells combined with macroporous hydroxyapatite scaffolds

results in substantial improvement in repairing large defects in long bones. Similarly, Solchaga et al. [27] reported the use of a combination of scaffold and bone marrow to treat osteochondral defects in rabbits. The results indicate that bone marrow loading appears to accelerate the first stage of the repair process after 3 weeks. In a caprine model of osteoarthritis, it has been demonstrated that local delivery of MSCs to the injured joints can stimulate marked regeneration of the medial meniscus, the implanted MSCs can be detected in the newly formed tissues, and the normally seen progressive destruction in this model is retarded [28]. The effect of systemically injected MSCs on fracture healing is evaluated in a mouse fracture healing model; the results indicate that MSCs transplantation significantly increases cartilage and bone content and improves the fracture healing by improving the callus biomechanical properties [29]. To treat femoral head necrosis in rabbit models, intravenous transplantation of MSCs has been proven to migrate directionally and survive in the necrotic femoral heads without immunological rejection [30].

Most of the studies were conducted using bone marrow- or other tissue-derived MSCs. However, the application of PB-MSCs is rarely reported. The main reason is that isolation and culture of PB-MSCs is not as easy as other tissue derived-MSCs. In 2006, our group demonstrated that PB-MSCs could enhance bone regeneration in the rabbit ulna critical-sized bone defect model [31].

Although the application of MSCs to cure a broad spectrum of diseases is promising, the efficacy still needs to be improved, especially for the differentiation of MSCs into specified functional cells *in vivo*. Many genes, chemicals, and cytokines have been used to enhance the efficacy of transplanted MSCs recently. For example, administration of interleukin 6 has been found to enhance the ability of MSCs to repair liver after CCL₄-induced fibrosis in mice [32]. Systemically administrated MSCs transduced with BMP2 and α 4 integrin have been demonstrated to increase bone density in a mouse model of osteopenia [33]. To manipulate specific gene expression in MSCs that may enhance their

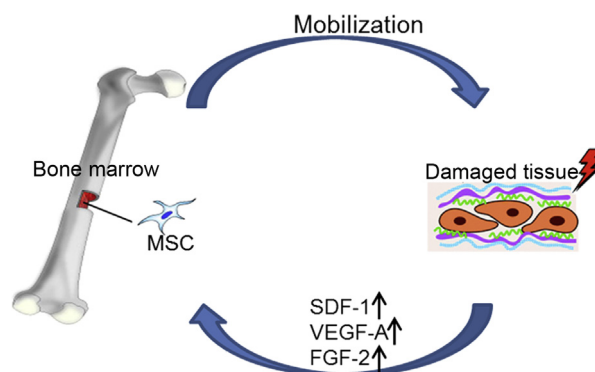


Figure 2 Schematic illustration of the migration of mesenchymal stem cells (MSCs) to the site of injury. MSCs reside in the bone marrow until stimulated by alarm signals, such as stromal cell-derived factor-1 (SDF1), vascular endothelial growth factor A (VEGF-A), and fibroblast growth factor 2 (FGF-2) in the plasma.

differentiation, proliferation, migration, and survival would be the future research directions.

Mobilization of MSCs

As mentioned, the characteristics of BM-MSCs and PB-MSCs are similar. Although BM-MSCs can be isolated and cultured very easily from bone marrow to fulfil the requirement of clinical use, the process of isolating MSCs from patients is complicated and painful. Compared with BM-MSCs, the number of PB-MSCs is rarer in the blood and the isolation method is not well defined. For clinical application, the PB-MSCs seem to be more promising as long as we can find the solutions to effectively isolate and culture them *in vitro*. The general method used to isolate PB-MSCs is Ficoll-Paque, which may affect the proliferation and survival of PB-MSCs, and the components of the medium also need to be evaluated, such as supplements of some growth factors.

Circulating MSCs are present in the peripheral blood in minimal concentrations under normal conditions. However, their numbers significantly increase in the blood after injury, such as bone fracture [34] and large acute burns [35]. Also, the increase in circulating MSCs is seen in patients with some chronic diseases, such as osteoporosis [36], breast cancer [11], and bone sarcomas [6]. It is believed that these increased MSCs may be released from the bone marrow. Our previous work has demonstrated that some osteoblasts involved in fracture healing are systemically mobilized and recruited to the fracture site from remote bone marrow, rather than passively leaked into the circulation and to the bone injury site [37]. How do MSCs in the bone marrow know where and when injuries happen? It is well known that various cytokines and a complex interaction of chemical alarm signals are released from the injured tissues, and these alarm signals are sent to MSCs through some pathways that have not yet been clearly elucidated. For example, significant increases of SDF1,

Table 1 Summary of publications using allogeneic mesenchymal stem cells (MSCs) in cell therapy applications.

Diseases/animal model	Recipient species	Outcome	Refs
Sjögren syndrome	Mouse, human	MSCs suppressed autoimmunity and restored salivary gland secretory function in both mouse models and Sjögren syndrome patients.	[47]
Ischaemic cardiomyopathy	Human	Allogeneic MSCs are as safe and effective as autologous MSCs in patients with left ventricular dysfunction due to ischaemic cardiomyopathy.	[46]
Drug-resistant polymyositis and dermatomyositis	Human	MSCs transplantation is safe and effective in patients with drug-resistant polymyositis or dermatomyositis.	[48]
Femoral segmental defect	Rat	Bone morphogenetic protein 2 (BMP-2) engineered allogeneic MSCs can repair critical bone defects to the same degree as rats treated with BMP-2 engineered autologous MSCs.	[49]
Spinal cord injury	Human	Neuropathic pain subsided from intermittent 10/10 to once/week 3/10 VAS. Recovery of muscle, bowel and sexual function was noted, along with a decrease in American spinal injury association (ASIA) score to "D".	[50]
Physéal bone bridge	Rabbit	No significant difference was found between rabbits with transplanted autogenous MSCs and rabbits with transplanted allogeneic MSCs either in the femur length, or in its valgus deformity.	[51]
Osteogenesis imperfect	Human	MSCs can engraft after transplantation, differentiate to osteoblasts as well as skin fibroblasts, and produce clinical benefits.	[52]
Acute myocardial infarction	Sheep	MSCs reduce infarct size and prevent subsequent adverse cardiac remodelling.	[53]
Multiple sclerosis	Human	The disease course was stabilized after the transplantation.	[54]
Systemic lupus erythematosus	Human	MSCs transplantation resulted in the induction of clinical remission and improvement in organ dysfunction in drug-resistant systemic lupus erythematosus patients. No transplantation-related adverse event was observed.	[55]
Cartilage defects	Pig	Quantification analyses for arthroscopy, histology, and magnetic resonance imaging revealed a better outcome in the MSCs-treated knees.	[56]
Acute myocarditis	Rat	The allogeneic administration of Fetal membrane-derived MSCs (FM-MSC) attenuated myocardial dysfunction and inflammation, and the host cell-mediated immune response was attenuated.	[57]
Recessive dystrophic epidermolysis bullosa	Human	MSCs administration resulted in an improvement in the skin of two patients, and the observed clinical benefit lasted for 4 months.	[58]
Ulcerative colitis	Human	Reduce the clinical and morphological indices of inflammatory activity in 34 (72.7%) patients.	[59]
Acute kidney injury	Rat	Reduce late renal fibrosis and loss of renal function in surviving animals.	[60]

Vascular endothelial growth factor A (VEGF-A), and Fibroblast growth factor 2 (FGF-2) in the plasma have been detected in patients with ST elevation acute myocardial infarction [38]. When MSCs in the bone marrow received these alarm signals, some of these can be mobilized into the bloodstream depending on how serious the injuries are, as illustrated in Fig. 2.

Administration of some cytokines can mimic the alarm signals and mobilize MSCs into blood. This can increase the number of MSCs in the blood, and makes the isolation of circulating MSCs possible. Recently, researchers have found that granulocyte-colony stimulating factor (G-CSF), a cytokine that contributes to haematopoietic progenitor cells and endothelial progenitor cells mobilization [39–41], can also be used to mobilize MSCs [42]. By using fibrin microbeads that can bind matrix-dependent cells, Kassis et al. [42] have isolated PB-MSCs from adult healthy human donors treated with G-CSF. Although they only get PB-MSCs from eight out of 11 samples, and the yield is lower than bone marrow, they made a big progress compared with the conventional method. In 2011, Iwasaki et al. [43] reported that the hepatocyte growth factor could also stimulate the migration of circulating mesoangioblasts (circulating mesenchymal cells that co-express endothelial makers).

Other issues of circulating MSCs: autologous versus allogeneic transplantation

Studies on circulating MSCs are rare in the literature. To our best understanding, circulating MSCs are a special subset of MSCs that are found in the circulation. However, their origin may be from bone marrow and other pools of MSCs in the body, and their release is tightly controlled by systemic and local factors (such as inflammatory cytokines, hormones, and growth factors). Stem cell homing refers to the inherent ability of stem cells to navigate toward a specific location (such as injury, inflammation, or infection) through unfamiliar areas. Stem cell mobilization means stem cells assemble and move toward specific stimuli or signals (directional movement), whereas cell migration is the inherent ability of cell movement and the migration may be directional or random. There are debates on the use of autologous and allogeneic MSCs through local and systemic administration for cell therapy applications. When administered systemically, MSCs may share certain characters with circulating MSCs. Because circulating MSCs are difficult to obtain from the blood, currently, our knowledge about them comes largely from studies of BM-MSCs.

Autologous MSCs transplantation means that the patient receives their own MSCs, with no risk of graft-versus-host disease. The disadvantage of autologous MSCs transplantation is that a longer time is needed (several days or weeks) for MSCs cell preparation, and the treatment window may be missed. In addition, sometimes the quality of autologous MSCs cannot be guaranteed in patients with comorbidities or advanced age [44,45].

Allogeneic MSCs transplantation is a procedure in which the patient receives MSCs from other donors. Allogeneic MSCs can be used as an “off-the-shelf” therapeutic agent, thus avoiding the painful bone marrow aspiration and culture delays prior to treatment. The potential risk from

using allogeneic MSCs is rejection. However, so far, there have been no reports of serious adverse side effects following allogeneic systemic and local administration of MSCs, including immunological complications in animals and human studies reported. There is increasing evidence to prove that MSCs are immunosuppressive cells and that allogeneic MSCs may be used with similar therapeutic efficacy to autologous MSCs. The study carried out by Hare et al. [46] demonstrated that allogeneic MSCs are as safe and as effective as autologous MSCs in patients with ischaemic cardiomyopathy. They also found that allogeneic MSCs did not stimulate significant donor-specific alloimmune reactions. So, allogeneic MSCs transplantation seems to be more promising, as it ensures that patients can get treatment at the best time with well-prepared allogeneic MSCs. Publications showing administration of allogeneic MSCs for curing various diseases are summarized in Table 1.

To date, no standard procedure for therapeutic MSC administration has been agreed on, which remains one of the critical issues for the clinical application of MSCs. For instance, the following questions still have open answers. How many MSCs should be used for patients with different diseases? When is the best time for MSC application? What kind of MSCs is most suitable? Which routine of administration and how often should MSCs be given (once/week or twice/week)? All these issues are valid ones and should be addressed properly through carefully designed animal and clinical trials before large-scale clinical applications of MSCs can be trialled and accepted in patients with certain disease conditions.

Conclusion

In conclusion, MSCs play an important role in the regenerative processes of many tissues. Circulating MSCs is a new cell source for tissue regeneration and tissue engineering. The implication of circulating MSCs in diseases is more promising, although many questions remain to be clarified, such as MSC mobilization from the bone marrow, homing mechanisms to specific tissues, and functional roles *in vivo*. In clinical practice, the use of allogeneic MSCs is more practical and may become the main cell source for clinical cell therapy. There are still many questions needing answers, and these answers have to come from carefully designed animal and clinical trials.

Conflicts of interest

The authors indicate no potential conflicts of interest.

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References

- [1] Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, et al. Multilineage potential of adult human mesenchymal stem cells. *Science* 1999;284:143–7.
- [2] Jiang YH, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, et al. Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature* 2002;418:41–9.
- [3] Tse WT, Beyer W, Pendleton JD, D'Andrea A, Guinan EC. Bone marrow derived mesenchymal stem cells suppress T cell activation without inducing allogeneic anergy. *Blood* 2000;96:241a.
- [4] Paul J. Establishment of permanent cell strains from human adult peripheral blood. *Nature* 1958;182:808.
- [5] Luria EA, Panasyuk AF, Friedenstein AY. Fibroblast colony formation from monolayer cultures of blood cells. *Transfusion* 1971;11:345–9.
- [6] Bian ZY, Li G, Gan YK, Hao YQ, Xu WT, Tang TT. Increased number of mesenchymal stem cell-like cells in peripheral blood of patients with bone sarcomas. *Arch Med Res* 2009;40:163–8.
- [7] Lan Y, Kodati S, Lee HS, Omoto M, Jin Y, Chauhan SK. Kinetics and function of mesenchymal stem cells in corneal injury. *Invest Ophthalmol Vis Sci* 2012;53:3638–44.
- [8] He Q, Wan C, Li G. Concise review: multipotent mesenchymal stromal cells in blood. *Stem Cells* 2007;25:69–77.
- [9] Castromalaspina H, Gay RE, Resnick G, Kapoor N, Meyers P, Chiarieri D, et al. Characterization of human-bone marrow fibroblast colony-forming cells (Cfu-F) and their progeny. *Blood* 1980;56:289–301.
- [10] Kuznetsov SA, Mankani MH, Gronthos S, Satomura K, Bianco P, Robey PG. Circulating skeletal stem cells. *J Cell Biol* 2001;153:1133–40.
- [11] Fernandez M, Simon V, Herrera G, Cao C, Del Favero H, Minguell JJ. Detection of stromal cells in peripheral blood progenitor cell collections from breast cancer patients. *Bone Marrow Transpl* 1997;20:265–71.
- [12] Zvaifler NJ, Marinova-Mutafchieva L, Adams G, Edwards CJ, Moss J, Burger JA, et al. Mesenchymal precursor cells in the blood of normal individuals. *Arthritis Res* 2000;2:477–88.
- [13] Elfenbein GJ, Sackstein R. Primed marrow for autologous and allogeneic transplantation: a review comparing primed marrow to mobilized blood and steady-state marrow. *Exp Hematol* 2004;32:327–39.
- [14] Brooke G, Tong H, Levesque JP, Atkinson K. Molecular trafficking mechanisms of multipotent mesenchymal stem cells derived from human bone marrow and placenta. *Stem Cells Dev* 2008;17:929–40.
- [15] Kollar K, Cook MM, Atkinson K, Brooke G. Molecular mechanisms involved in mesenchymal stem cell migration to the site of acute myocardial infarction. *Int J Cell Biol* 2009;2009:904682.
- [16] Wynn RF, Hart CA, Corradi-Perini C, O'Neill L, Evans CA, Wraith JE, et al. A small proportion of mesenchymal stem cells strongly expresses functionally active CXCR4 receptor capable of promoting migration to bone marrow. *Blood* 2004;104:2643–5.
- [17] Li Y, Yu X, Lin S, Li X, Zhang S, Song YH. Insulin-like growth factor 1 enhances the migratory capacity of mesenchymal stem cells. *Biochem Biophys Res Commun* 2007;356:780–4.
- [18] Song C, Li G. CXCR4 and matrix metalloproteinase-2 are involved in mesenchymal stromal cell homing and engraftment to tumors. *Cytotherapy* 2011;13:549–61.
- [19] Ruster B, Gottig S, Ludwig RJ, Bistrrian R, Muller S, Seifried E, et al. Mesenchymal stem cells display coordinated rolling and adhesion behavior on endothelial cells. *Blood* 2006;108:3938–44.
- [20] Sackstein R, Merzaban JS, Cain DW, Dagia NM, Spencer JA, Lin CP, et al. *Ex vivo* glycan engineering of CD44 programs human multipotent mesenchymal stromal cell trafficking to bone. *Nat Med* 2008;14:181–7.
- [21] Shi M, Li J, Liao L, Chen B, Li B, Chen L, et al. Regulation of CXCR4 expression in human mesenchymal stem cells by cytokine treatment: role in homing efficiency in NOD/SCID mice. *Haematologica* 2007;92:897–904.
- [22] Tsai LK, Leng Y, Wang ZF, Leeds P, Chuang DM. The mood stabilizers valproic acid and lithium enhance mesenchymal stem cell migration via distinct mechanisms. *Neuropsychopharmacol* 2010;35:2225–37.
- [23] Cheng Z, Ou L, Zhou X, Li F, Jia X, Zhang Y, et al. Targeted migration of mesenchymal stem cells modified with CXCR4 gene to infarcted myocardium improves cardiac performance. *Mol Ther* 2008;16:571–9.
- [24] Saito T, Kuang JQ, Bittira B, Al-Khalidi A, Chiu RC. Xenotransplant cardiac chimera: immune tolerance of adult stem cells. *Ann Thorac Surg* 2002;74:19–24. discussion 24.
- [25] Stamm C, Westphal B, Kleine HD, Petzsch M, Kittner C, Klinge H, et al. Autologous bone-marrow stem-cell transplantation for myocardial regeneration. *Lancet* 2003;361:45–6.
- [26] Quarto R, Mastrogiacomo M, Cancedda R, Kutepov SM, Mukhachev V, Lavroukov A, et al. Repair of large bone defects with the use of autologous bone marrow stromal cells. *New Engl J Med* 2001;344:385–6.
- [27] Solchaga LA, Gao JZ, Dennis JE, Awadallah A, Lundberg M, Caplan AI, et al. Treatment of osteochondral defects with autologous bone marrow in a hyaluronan-based delivery vehicle. *Tissue Eng* 2002;8:333–47.
- [28] Murphy JM, Fink DJ, Hunziker EB, Barry FP. Stem cell therapy in a caprine model of osteoarthritis. *Arthritis Rheum* 2003;48:3464–74.
- [29] Granero-Molto F, Weis JA, Miga MI, Landis B, Myers TJ, O'Rear L, et al. Regenerative effects of transplanted mesenchymal stem cells in fracture healing. *Stem Cells* 2009;27:1887–98.
- [30] Li ZH, Liao W, Cui XL, Zhao Q, Liu M, Chen YH, et al. Intravenous transplantation of allogeneic bone marrow mesenchymal stem cells and its directional migration to the necrotic femoral head. *Int J Med Sci* 2011;8:74–83.
- [31] Wan C, He Q, Li G. Allogenic peripheral blood derived mesenchymal stem cells (MSCs) enhance bone regeneration in rabbit ulna critical-sized bone defect model. *J Orthop Res* 2006;24:610–8.
- [32] Nasir GA, Mohsin S, Khan M, Shams S, Ali G, Khan SN, et al. Mesenchymal stem cells and interleukin-6 attenuate liver fibrosis in mice. *J Transl Med* 2013;11:78.
- [33] Kumar S, Nagy TR, Ponnazhagan S. Therapeutic potential of genetically modified adult stem cells for osteopenia. *Gene Ther* 2010;17:105–16.
- [34] Alm JJ, Koivu HM, Heino TJ, Hentunen TA, Laitinen S, Aro HT. Circulating plastic adherent mesenchymal stem cells in aged hip fracture patients. *J Orthop Res* 2010;28:1634–42.
- [35] Mansilla E, Marin GH, Drago H, Sturla F, Salas E, Gardiner C, et al. Bloodstream cells phenotypically identical to human mesenchymal bone marrow stem cells circulate in large amounts under the influence of acute large skin damage: new evidence for their use in regenerative medicine. *Transpl Proc* 2006;38:967–9.

- [36] Dalle Carbonare L, Valenti MT, Zanatta M, Donatelli L, Lo Cascio V. Circulating mesenchymal stem cells with abnormal osteogenic differentiation in patients with osteoporosis. *Arthritis Rheum* 2009;60:3356–65.
- [37] Shirley D, Marsh D, Jordan G, McQuaid S, Li G. Systemic recruitment of osteoblastic cells in fracture healing. *J Orthop Res* 2005;23:1013–21.
- [38] Wang Y, Johnsen HE, Mortensen S, Bindslev L, Ripa RS, Haack-Sorensen M, et al. Changes in circulating mesenchymal stem cells, stem cell homing factor, and vascular growth factors in patients with acute ST elevation myocardial infarction treated with primary percutaneous coronary intervention. *Heart* 2006;92:768–74.
- [39] Aicher A, Zeiher AM, Dimmeler S. Mobilizing endothelial progenitor cells. *Hypertension* 2005;45:321–5.
- [40] Takahashi T, Kalka C, Masuda H, Chen D, Silver M, Kearney M, et al. Ischemia- and cytokine-induced mobilization of bone marrow-derived endothelial progenitor cells for neovascularization. *Nat Med* 1999;5:434–8.
- [41] Paganessi LA, Walker AL, Tan LL, Holmes I, Rich E, Fung HC, et al. Effective mobilization of hematopoietic progenitor cells in G-CSF mobilization defective CD26^{-/-} mice through AMD3100-induced disruption of the CXCL12-CXCR4 axis. *Exp Hematol* 2011;39:384–90.
- [42] Kassis I, Zangi L, Rivkin R, Levinsky L, Samuel S, Marx G, et al. Isolation of mesenchymal stem cells from G-CSF-mobilized human peripheral blood using fibrin microbeads. *Bone Marrow Transpl* 2006;37:967–76.
- [43] Iwasaki M, Koyanagi M, Kossman H, Monsefi N, Rupp S, Trauth J, et al. Hepatocyte growth factor mobilizes non-bone marrow-derived circulating mesoangioblasts. *Eur Heart J* 2011;32:627–36.
- [44] Zhuo Y, Li SH, Chen MS, Wu J, Kinkaid HY, Fazel S, et al. Aging impairs the angiogenic response to ischemic injury and the activity of implanted cells: combined consequences for cell therapy in older recipients. *J Thorac Cardiovasc Surg* 2010;139:1286–94.
- [45] Kinkaid HY, Huang XP, Li RK, Weisel RD. What's new in cardiac cell therapy? Allogeneic bone marrow stromal cells as "universal donor cells". *J Card Surg* 2010;25:359–66.
- [46] Hare JM, Fishman JE, Gerstenblith G, DiFede Velazquez DL, Zambrano JP, Suncion VY, et al. Comparison of allogeneic vs autologous bone marrow-derived mesenchymal stem cells delivered by transendocardial injection in patients with ischemic cardiomyopathy: the POSEIDON randomized trial. *JAMA* 2012;308:2369–79.
- [47] Xu J, Wang D, Liu D, Fan Z, Zhang H, Liu O, et al. Allogeneic mesenchymal stem cell treatment alleviates experimental and clinical Sjogren syndrome. *Blood* 2012;120:3142–51.
- [48] Wang D, Zhang H, Cao M, Tang Y, Liang J, Feng X, et al. Efficacy of allogeneic mesenchymal stem cell transplantation in patients with drug-resistant polymyositis and dermatomyositis. *Ann Rheum Dis* 2011;70:1285–8.
- [49] Tsuchida H, Hashimoto J, Crawford E, Manske P, Lou J. Engineered allogeneic mesenchymal stem cells repair femoral segmental defect in rats. *J Orthop Res* 2003;21:44–53.
- [50] Ichim TE, Solano F, Lara F, Paris E, Ugalde F, Rodriguez JP, et al. Feasibility of combination allogeneic stem cell therapy for spinal cord injury: a case report. *Int Arch Med* 2010;3:30.
- [51] Planka L, Gal P, Kecova H, Klima J, Hlucilova J, Filova E, et al. Allogeneic and autogenous transplantations of MSCs in treatment of the physeal bone bridge in rabbits. *BMC Biotechnol* 2008;8:70.
- [52] Horwitz EM, Gordon PL, Koo WK, Marx JC, Neel MD, McNall RY, et al. Isolated allogeneic bone marrow-derived mesenchymal cells engraft and stimulate growth in children with osteogenesis imperfecta: Implications for cell therapy of bone. *Proc Natl Acad Sci U S A* 2002;99:8932–7.
- [53] Houtgraaf JH, de Jong R, Kazemi K, de Groot D, van der Spoel TI, Arslan F, et al. Intracoronary infusion of allogeneic mesenchymal precursor cells directly following experimental acute myocardial infarction reduces infarct size, abrogates adverse remodeling and improves cardiac function. *Circ Res* 2013;113:153–66.
- [54] Liang J, Zhang H, Hua B, Wang H, Wang J, Han Z, et al. Allogeneic mesenchymal stem cells transplantation in treatment of multiple sclerosis. *Mult Scler* 2009;15:644–6.
- [55] Wang D, Zhang H, Liang J, Li X, Feng X, Wang H, et al. Allogeneic mesenchymal stem cell transplantation in severe and refractory systemic lupus erythematosus: 4 years experience. *Cell Transpl* 2012 [Epub ahead of print] <http://dx.doi.org/10.3727/096368912X658719>.
- [56] Nakamura T, Sekiya I, Muneta T, Hatsushika D, Horie M, Tsuji K, et al. Arthroscopic, histological and MRI analyses of cartilage repair after a minimally invasive method of transplantation of allogeneic synovial mesenchymal stromal cells into cartilage defects in pigs. *Cytotherapy* 2012;14:327–38.
- [57] Ishikane S, Yamahara K, Sada M, Harada K, Kodama M, Ishibashi-Ueda H, et al. Allogeneic administration of fetal membrane-derived mesenchymal stem cells attenuates acute myocarditis in rats. *J Mol Cell Cardiol* 2010;49:753–61.
- [58] Conget P, Rodriguez F, Kramer S, Allers C, Simon V, Palisson F, et al. Replenishment of type VII collagen and re-epithelialization of chronically ulcerated skin after intradermal administration of allogeneic mesenchymal stromal cells in two patients with recessive dystrophic epidermolysis bullosa. *Cytotherapy* 2010;12:429–31.
- [59] Lazebnik LB, Kniazev OV, Konopliannikov AG, Parfenov AI, Ruchkina IN, Mikhailova ZF, et al. Allogeneic mesenchymal stromal cells in patients with ulcerative colitis: two years of observation. *Eksp Klin Gastroenterol* 2010;3–15 [Article in Russian].
- [60] Togel F, Cohen A, Zhang P, Yang Y, Hu Z, Westenfelder C. Autologous and allogeneic marrow stromal cells are safe and effective for the treatment of acute kidney injury. *Stem Cells Dev* 2009;18:475–85.