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Review

Mesenchymal stem cells: a new trend for cell therapy

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Mesenchymal stem cells (MSCs), the major stem cells for cell therapy, have been used in the clinic for approximately 10 years. From animal models to clinical trials, MSCs have afforded promise in the treatment of numerous diseases, mainly tissue injury and immune disorders. In this review, we summarize the recent opinions on methods, timing and cell sources for MSC administration in clinical applications, and provide an overview of mechanisms that are significant in MSC-mediated therapies. Although MSCs for cell therapy have been shown to be safe and effective, there are still challenges that need to be tackled before their wide application in the clinic.

Introduction

Stem cells are unspecialized cells with the ability to renew themselves for long periods without significant changes in their general properties. They can differentiate into various specialized cell types under certain physiological or experimental conditions. Cell therapy is a sub-type of regenerative medicine. Cell therapy based on stem cells describes the process of introducing stem cells into tissue to treat a disease with or without the addition of gene therapy. Hematopoietic stem cells (HSCs) have been widely used for allogeneic cell therapy. The successful isolation of pluripotent embryonic stem (ES) cells from the inner cell mass of early embryos has provided a powerful tool for biological research. ES cells can give rise to almost all cell lineages and are the most promising cells for regenerative medicine. The ethical issues related to their isolation have promoted the development of induced pluripotent stem (iPS) cells, which share many properties with ES cells without ethical concerns. However, one key property of ES cells and iPS cells that may seriously compromise their utility is their potential for teratoma formation.

Due to the limitation of using ES and iPS cells in the clinic, great interest has developed in mesenchymal stem cells (MSCs), which are free of both ethical concerns and teratoma

formation. These cells were first isolated and characterized by Friedenstein and his colleagues in 1974. MSCs, also called mesenchymal stromal cells, are a subset of non-hematopoietic adult stem cells that originate from the mesoderm. They possess self-renewal ability and multilineage differentiation into not only mesoderm lineages, such as chondrocytes, osteocytes and adipocytes, but also ectodermic cells and endodermic cells[1–5]. MSCs exist in almost all tissues. They can be easily isolated from the bone marrow, adipose tissue, the umbilical cord, fetal liver, muscle, and lung and can be successfully expanded *in vitro*[6–10]. The number of clinical trials on MSCs has been rising since 2004 (Figure 1). Although the "gold rush" to use MSCs in clinical settings began with high enthusiasm in many countries, with China, Europe and US leading the way (http://clinicaltrial.cn), numerous scientific issues remain to be resolved before the establishment of clinical standards and governmental regulations.

What can MSCs do?

Currently, there are 344 registered clinical trials in different clinical trial phases (Figure 2) aimed at evaluating the potential of MSC-based cell therapy worldwide. With the advancement of preclinical studies, MSCs have been shown to be effective in the treatment of many diseases, including both immune diseases and non-immune diseases (Figure 3).

MSCs in tissue repair

The wide tissue distribution and multipotent differentiation of

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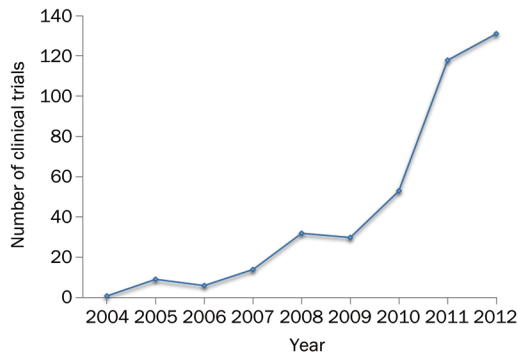E-mail drshaoli@sina.com (Li SHAO);
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Figure 1. Number of registered clinical trials of mesenchymal stem cells-based therapy on ClinicalTrials.gov.

- Phase I 31.4%
- Phase II 49.6%
- Phase III 15.3%
- Phase IV 3.7%
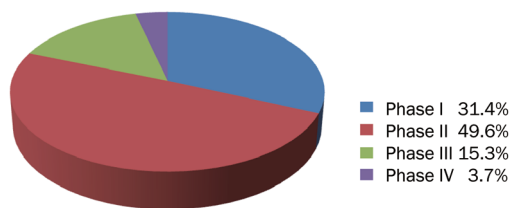
Figure 2. Clinical phages of mesenchymal stem cells-based therapy. Data from ClinicalTrials.gov.

- Myocardial infarction (22.9%)
- Graft versu host disease (16.0%)
- Diabetes (10.3%)
- Liver cirrhosis (10.3%)
- Spinal cord injury (9.2%)
- Osteoarthritis (8.0%)
- Crobn's disease (3.8%)
- Multiple scllerosis (3.4%)
- Aplastic anemia (1.5%)
- Systemic lupus (1.1%)
- Rheumatoid arthritis (1.1%)
- Parkinson's disease (0.8%)
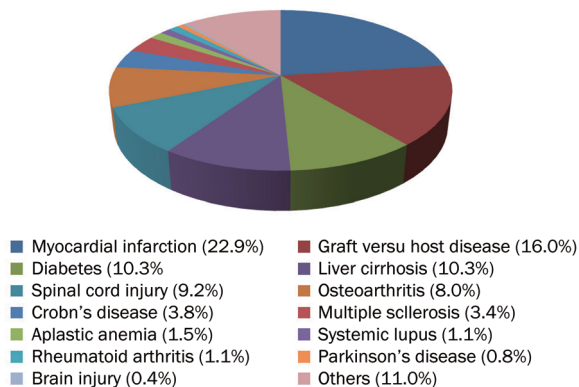- Brain injury (0.4%)
- Others (11.0%)

Figure 3. Percentages of the common diseases now treated with mesenchymal stem cells. Data from ClinicalTrials.gov.

MSCs together with the observed reparative effects of infused MSCs in many clinical and preclinical models[11–26] strongly suggest a critical role of MSCs in injury healing. They are believed to be responsible for growth, wound healing, and replacing cells that are lost through daily wear and tear and pathological conditions. Because of these functions, they have been shown to be effective in the treatment of tissue injury and degenerative diseases. In the digestive system, autologous bone marrow mesenchymal stem cells (BM-MSCs) improved the clinical indices of liver function in liver cirrhosis patients and liver failure patients caused by hepatitis B[27, 28]. BM-MSCs

can also exert strong therapeutic effects in the musculoskeletal system. They have been shown to be effective in the regeneration of periodontal tissue defects, diabetic critical limb ischemia, bone damage caused by osteonecrosis and burn-induced skin defects[29–31]. In preclinical studies, investigations by the Prockop team have also shown that human MSCs are effective in treating myocardial infarction[32] and cornea damage[33] through the secretion of tumor necrosis factor-inducible gene 6 protein (TSG-6), which reduces inflammation and promotes tissue reconstruction. A similar phenomenon has been reported for MSCs in treating other tissue injuries, such as the brain, spinal cord[34] and lung[35, 36], all target organs of MSCs in the future. Additionally, co-transplantation of MSCs can enhance the effect of HSCs in treating radiation victims[37].

MSCs in immune disorder therapy
In addition to their property of treating tissue injury, MSCs are also applied to alleviate immune disorders because MSCs have a powerful capacity of regulating immune responses. Various studies have evaluated the therapeutic effect of MSCs in preclinical animal models and demonstrated great clinical potential. For example, MSCs have been successfully applied to reverse graft-versus-host disease (GvHD) in patients receiving bone marrow transplantation[38, 39], especially in patients diagnosed with severe steroid resistance[40–42]. Similarly, in systemic lupus erythematosus (SLE) and Crohn's disease patients, both autologous and allogeneic MSCs were able to suppress inflammation and reduce damage to the kidneys and bowel through the possible induction of regulatory T cells in patients[43–46]. It also has been reported that BM-MSCs can improve multiple system atrophy (MSA)[47], multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS)[48–50], and stroke, likely through immediate immunomodulatory effects[51]. Osiris' Prochymal, the world's first stem cell drug approved in Canada on May 12, 2012, was successful in phase III clinical trials in treating GvHD and Crohn's disease and has become the only stem cell-based drug approved by FDA[40, 52].

Methods and techniques of applying MSCs in the clinic
Engraftment of MSCs
In all of the preclinical and clinical studies, the engraftment of MSCs into damaged tissues via migration to enhance tissue repair/regeneration is a crucial process for clinical efficacy, regardless of the type of organ or specific disease. As more and more clinical studies are performed, the engraftment properties of MSCs are gradually being evaluated in many models and clinical trials. In 2000, a study of human MSC in utero transplantation in sheep demonstrated long-term engraftment as long as 13 months after transplantation, even when cells were transplanted after the expected development of immunocompetence, and the transplanted human MSCs could undergo site-specific differentiation into chondrocytes, adipocytes, myocytes and cardiomyocytes, bone marrow stromal cells and thymic stroma[53]. However, the overwhelming majority of MSCs were found in the lung after systemic administration in normal recipients, and these MSCs disap-

peared gradually over time[54]. The mechanisms of these phenomena are still unclear.

The site of delivery most likely affects the trafficking of MSCs to target organs. Generally, two approaches of systemic administration have been used for MSC applications. One is intravenous injection, such as peripheral vein injection (tail vein in mice), utilizing the capabilities of MSCs to migrate to specific inflammatory tissues *in vivo*, including the cartilage, liver, and lung[55, 56]. Engraftment was demonstrated in animal models and was capable of persisting for as long as 13 months after transplantation[53]. The other approach is local intraarterial injection, which can enhance the accumulation and increase the dose of MSCs in injured tissues. In a phase I study of MSCs as a treatment for liver cirrhosis in 2007, patients were injected with human MSCs through liver arteries[57]. Moreover, to increase the number of MSCs and the efficiency of differentiation at the damaged sites, studies on liver cirrhosis, osteonecrosis, skin defects, and spinal cord injury employed local injections instead of the systemic administration of MSCs[31]. However, which route of administration is best for a particular disease and the possible contraindication of such clinical usage are still unknown.

Time of MSC administration

In addition to the different methods of administration, the timing of delivery and number of cells delivered are also very important. Sudres *et al* found in their study that MSCs failed to prevent GvHD in mice, and the failure was not due to MSC rejection[58]. Meanwhile, our finding showed that MSCs could prolong survival in a GvHD mouse model[59]. One difference between these two studies was the infusion time of MSCs[60]. Sudres *et al* injected MSCs 10–15 min before GvHD induction, whereas we injected MSCs 3 d and 7 d after bone marrow transplantation. It is possible that the time of MSC administration is important for the therapeutic effect. Based on the above discussion that the immunosuppressive ability of MSCs must be induced by inflammatory cytokines, it is conceivable that MSC administration at the peak of inflammation may improve the treatment effect. However, this hypothesis needs to be further tested.

Cell sources of MSCs

MSCs exist in almost all tissues. They can be easily isolated from the bone marrow, adipose tissue, umbilical cord, fetal liver, muscle, and lung and can be successfully expanded *in vitro*[6–10]. Although the major source of MSCs in clinic trials is umbilical cord, recent studies have suggested that the allogenicity of MSCs have no significant adverse impact on the engraftment of MSCs in wound healing[61]. It is better to use freshly isolated MSCs because it has been shown that 5 major histocompatibility complex (MHC II) molecules could be increased during *in vitro* expansion[62, 63].

Therapeutic mechanisms of MSCs

As mentioned above, MSCs have displayed great potential in treating a large number of immune and non-immune diseases.

However, there are still major questions concerning the optimal dosage of MSCs, routes of administration, best engraftment time and the fate of the cells after infusion[64]. Thus, it is critical to explore the mechanisms governing MSC-based therapies. Although a uniform mechanism has not yet been discovered, the available data have revealed several working models for the beneficial effects of MSCs. Based on the current understanding, we summarize some key mechanisms that are significant in MSC-mediated therapies. It is noteworthy that for a given disease, multiple mechanisms are likely to contribute coordinately to the therapeutic effect of MSCs.

Homing efficiency

MSCs have a tendency to home to damaged tissue sites. When MSCs are delivered exogenously and systemically administered to humans and animals, they are always found to migrate specifically to damaged tissue sites with inflammation[65, 66], although many of the intravenously administered MSCs are trapped in the lung[67, 68]. The inflammation-directed MSC homing has been demonstrated to involve several important cell trafficking-related molecules: chemokines, adhesion molecules, and matrix metalloproteinases (MMPs). Among these chemokines, the chemokine (C-X-C motif) ligand 12- chemokine (C-X-C motif) receptor 4 and chemokine (C-C motif) ligand 2- chemokine (C-C motif) receptor 2 axes are most studied[69, 70]. Accordingly, CXCR4 was transduced into MSCs to improve their *in vivo* engraftment and therapeutic efficacy in a rat myocardial infarction model[71]. The adhesion molecule P-selectin and the VCAM-1 (vascular cell adhesion protein 1)-VLA-4 (very late antigen-4) interaction has been shown to be key mediators in MSC rolling and firm adherence to endothelial cells *in vitro* and *in vivo*[72]. Interestingly, in a recent report, VCAM-1 antibody-coated MSCs exhibited a higher efficiency of engraftment into inflamed mesenteric lymph nodes and the colon than uncoated MSCs in a mouse inflammatory bowel disease (IBD) model[73], suggesting that modulations of the homing property of MSCs could be a viable approach in enhancing their therapeutic effectiveness. In addition to chemokines and adhesion molecules, several MMPs, such as MMP-2 and membrane type 1 MMP (MT1-MMP), have been shown to be essential in the invasiveness of MSCs[74, 75]. It is worth noting that all the homing-related molecules can be up-regulated by inflammatory cytokines, such as TNF and IL-1[76, 77]. Therefore, different inflammation statuses (*ie*, different levels of inflammatory cytokines) might lead to distinct MSC engraftment and therapeutic efficiencies.

Tumors can be regarded as wounds that never heal and continuously generate various inflammatory cytokines[78]. Indeed, MSCs that are either *de novo* mobilized or exogenously administered have been found to migrate to tumors and adjacent tissue sites[79]. In view of this property, approaches have been developed to engineer several tumor-killing agents, such as IFNα, IFNβ, IL-12, and TNF-related apoptosis-inducing ligand (TRAIL), in MSCs for tumor-targeted therapy in animal models[80–84]. More recently, MSCs have also been undergoing development as vehicles for the delivery of nanoparticles to

enhance their tumoricidal effects[85, 86]. Further investigations in this direction may lead to novel therapeutic strategies for cancer.

Differentiation potential and tissue engineering

As typical multipotent stem cells, MSCs have been shown to possess the capability to differentiate into a variety of cell types, including adipocytes, osteoblasts, chondrocytes, myoblasts and neuron-like cells. Although it is currently believed that the therapeutic benefits of MSCs are due to more complicated mechanisms, they have been indicated to be able to differentiate into osteoblasts, cardiomyocytes and other tissue-specific cells after their *in vivo* systemic infusion in the treatment of osteogenesis imperfecta and myocardial infarction in both animals and humans[53, 87, 88].

In addition to systemic delivery, MSCs can be delivered together with various natural and synthetic biomaterial scaffolds. Either undifferentiated or differentiated MSCs can be loaded onto scaffolds before their implantation into damaged tissue sites[89, 90]. Such technologies have been successfully applied in cartilage repair and long bone repair, with the generation of well-integrated and functional hard tissues[91, 92]. The advantage of a tissue-engineered MSC delivery system lies in the ease of controlling and manipulating the implanted cells and tissues, with reduced side effects impacting other organs and tissues. Current improvements in delivery vehicles and compatibility between the scaffolds and MSCs will help to develop a mature technology for clinical applications.

Production of trophic factors

Accumulating evidence has revealed that the therapeutic benefits of MSCs are largely dependent on their capacity to act as a trophic factor pool. After MSCs home to damaged tissue sites for repair, they interact closely with local stimuli, such as inflammatory cytokines, ligands of Toll-like receptors (TLRs) and hypoxia, which can stimulate MSCs to produce a large amount of growth factors that perform multiple functions for tissue regeneration[93–95]. Many of these factors are critical mediators in angiogenesis and the prevention of cell apoptosis, such as vascular endothelial growth factor (VEGF), insulin-like growth factor 1 (IGF-1), basic fibroblast growth factors (bFGF), hepatocyte growth factor (HGF), IL-6 and CCL-2[94, 96, 97]. Interestingly, a recent study found that the therapeutic effect of neuronal progenitors on EAE was solely dependent on leukemia inhibitory factor (LIF), revealing a similar trophic function as other tissue progenitors/stem cells[98]. Moreover, many reports have demonstrated that pre-treatment with growth factors or gene modification of MSCs can enhance the therapeutic efficacy for myocardial infarction and other wound-healing processes[99, 100]. A further understanding of the molecular pathways involved in growth factor production will be helpful to develop better strategies for MSC-based therapies.

Immunomodulation

In the last few years, MSCs have been shown to be effective in treating various immune disorders in human and animal models. In both *in vitro* and *in vivo* studies, MSCs have been shown to suppress the excessive immune responses of T cells, B cells, dendritic cells, macrophages, and natural killer cells[101, 102]. The underlying mechanisms are believed to be a combined effect of many immunosuppressive mediators. A majority of the mediators are inducible by inflammatory stimuli, such as nitric oxide (NO), indoleamine 2,3, dioxygenase (IDO), prostaglandin E2 (PGE2), tumor necrosis factor-inducible gene 6 protein (TSG6), CCL-2, and programmed death ligand 1 (PD-L1)[68, 103–108]. These factors are minimally expressed in unactivated MSCs unless they are stimulated by several inflammatory cytokines, such as IFNγ, TNFα, and IL-1[78, 103]. The neutralization of either immunosuppressive effectors or inflammatory cytokines could reverse MSC-mediated immunosuppression[79]. The concept of inflammation-licensed immunosuppression favors a more rational design for the clinical use of MSCs. First, an optimal administration time point should be carefully selected according to the levels and ratios of different cytokines in the body during disease progression. Previous reports have demonstrated that MSC administration after disease onset may be better than at the same time of disease induction in a mouse GvHD model[71, 79]. Second, cytokine priming should be attempted to improve the therapeutic effect of MSCs. Cheng *et al* reported that IFNγ-pretreated MSCs protected 100% of mice from GvHD-induced death[71]. Third, the therapeutic efficacy of MSCs most likely depends on the nature of different diseases due to the distinct inflammatory environments. Even for a specific disease, the diversity of microenvironments in different tissues may also produce different curative effects of MSCs. Therefore, the precise *in vivo* mechanism of MSCs may be more complex than observed *in vitro*. Further defining such mechanisms will help to develop better strategies for the clinical use of MSCs.

Unsolved problems and challenges

Although significant progress has been made in stem cell research in recent years, cell therapy with stem cells is far from a mature clinical technology. Because they are free of ethical concerns and have numerous sources, low immunogenicity and no teratoma risk, MSCs are the most commonly used stem cells in current clinical applications. However, there are still several major hurdles to their widespread utility. Further research is needed on interactions between MSCs and the inflammatory milieu in which they reside and the therapeutic mechanisms of MSCs. Furthermore, it is still not known which source should be used for which disease, which route of administration is best suited for a particular disease, and possible contraindications to their clinical use. Once administered, the parameters for monitoring clinical effectiveness also need to be established and are likely to vary for different disorders. Most importantly, established standards for cell expansion protocols, product quality, and safety controls are not available in most countries. Government regulatory agencies are eagerly waiting for detailed answers to these questions to establish regulatory polices to meet the challenges of this newly emerging and rapidly advancing field and benefit

patients suffering a wide array of diseases. We look forward to using soluble products of MSCs instead of MSCs themselves in the future, which may simplify the administration of cells and make it safer.

Acknowledgements

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