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Review

MSCs: Biological characteristics, clinical applications and their outstanding concerns

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ABSTRACT

Mesenchymal stem cells (MSCs) are multi-potent adult stem cells harboring multi-lineage differentiation potential and immunosuppressive properties that make MSCs an ideal candidate cell type for immunomodulation and regenerative medicine. Currently, MSC-related researches and clinical trials have evoked exciting promise in a variety of disorders and tissue regeneration. However, it must be recognized that several critical potential problems have also emerged from current clinical trials, for example: (1) the indefinite association between the phenotypic characteristics and the biological functions of MSCs; (2) the lack of clinical data to support the long-term safety of MSCs; (3) the need for further clarification of multiple mechanisms of MSC transplant actions *in vivo*; and (4) the lack of comparability of MSC transplant efficacy. Therefore, MSC-based therapies could not yet be considered a routine treatment in the clinic. Based on these, we proposed that large-scale and multi-center clinical trials of MSC-based therapies should be initiated under strict supervision. These interventions might help to establish a new clinical paradigm to turn MSC transplantation into a routine therapy for at least some diseases in the near future.

1. Introduction

Mesenchymal stem cells (MSCs) are a heterogeneous subset of stromal stem cells that have the ability of self-renewal and multipotency, which could differentiate into cells of the mesodermal lineages and other embryonic lineages, including adipocytes, osteocytes, chondrocytes, hepatocytes, neurons, muscle cells, epithelial cells, etc. (Pittenger et al., 1999; Jiang et al., 2002; Lee et al., 2004). Moreover, an increasing number of evidence indicates that MSCs possess potential immunomodulation, anti-inflammation properties, and trophic effects (Nauta and Fibbe, 2007; Oh et al., 2008; Ankrum and Karp, 2010). Compared with embryonic stem cells (ESCs) and other tissue-specific stem cells such as hematological and neural stem cells, MSCs have several advantages, such as easy availability as well as few ethical concerns and low immunogenicity. In addition, they also have extensively proliferative properties *in vitro* while maintaining their undifferentiated multi-potent status (Meirelles Lda et al., 2008). These properties make MSCs an ideal candidate cell type for tissue engineering, regenerative medicine and autoimmune disease treatment.

Several clinical reports on MSC-based disease treatment have been published in the past decade, and evoked great excitement and therapeutic promises for several diseases. As early as in the 1990s, cultured MSCs have already been infused to reduce acute and chronic graft-versus-host disease (GVHD) among patients receiving allogenic haematopoietic stem cell transplantation, and to ameliorate symptoms among patients with osteogenesis imperfecta and glycogen storage disease (Bordignon et al., 1999; Horwitz et al., 2002; Koc and Lazarus, 2001). In recent years, several other small-scale clinical trials focusing on a variety of diseases, including myocardial infarcts, diabetes, different types of neurological disorders, and systemic lupus erythematosus (Phinney and Prockop, 2007; Song et al., 2010; Mizuno, 2009), have been conducted. An increasing number of data has showed that the therapeutic effects of MSCs not only rely on their differentiation ability to repair damaged tissue, but also depend on their potency to modulate local environment, activate endogenous progenitor cells, and secrete various factors (Zhang et al., 2007; Togel et al., 2007).

However, the detailed mechanisms of these effects are far from clear, and it must be recognized that there are several outstanding concerns in therapeutic application of MSCs. Several biologists have stressed that MSC-based therapies remain poorly understood, and the scientific evidence about their safety and efficacy in clinical trials is needed. Taken together, the status quo of MSC-based therapies indicates that MSC transplantation could not yet be considered a routine approach in the clinic, and large-scale and multi-center MSC-based clinical trials are urgently required.

In this review, we have summarized the current researches pertaining to the biological characteristics and the current small-scale

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Table 1
Cell markers of MSCs was classified by different types.

Marker type		Positive (+)	Negative (−)
Multi-lineage cell molecular	Haematopoietic progenitors	CD117 (c-Kit); CD133	CD34; HLA-DR
	Monocyte/macrophage		CD14
	Leukocyte	CD10	CD45; CD6
	Endothelial	CD146	CD31(PECAM);Muc-18; VIII-Factor-associated antigen
	Pericytes	SG5	
	Mesenchymal stromal	CD9; CD73 (SH3); CD105 (SH2); Stro-1; CD54; CD90(Thy-1); Vimentin	
	Osteogenic	Osteonectin;Osteopontin; Osteocalcin	Bone sialoprotein
	Adipogenic	CEBPα	PPARγ2
	Smooth muscle	α-Smooth muscle actin	
	Skeletal muscle		MyoD
	Fibroblastic	Type I/III/VI collagen; Fibronectin; Fibrin; β1 integrin subunit	
	Nerve	CD271; neuregulin	Neurofilament
Migration relative molecular	Selectin	P-selectin; CD166(VCAM-1); ICAM-1	L-selectin; E-selectin
	Integrins	CD10; CD29; CD44; CD49a; CD61/51; CD71	
	Chemokine receptor	CD184(CXCR4);	
	Selectin	P-selectin; CD166(VCAM-1)	L-selectin; E-selectin
Immune phenotype		MHC-I	MHC-II; CD40; CD80 (B7-1); CD86 (B7-2)
Others		SSEA-1; SSEA-4	

therapeutic applications of MSCs. Based on these, we have raised several potential concerns that should be clarified or resolved in the future before MSC transplantation could be considered a routine therapeutic approach in the clinic.

2. Biological characteristics of MSCs

2.1. Tissue distribution and sources of hMSCs

MSCs were originally isolated from bone marrow (BM) by Friedenstein and colleagues more than 40 years ago (Friedenstein et al., 1968), and have ever been named as fibroblast colony-forming units (CFU-Fs) or marrow stromal cells. They are currently termed as "mesenchymal stem cells" based on their property of differentiating into a variety of mesodermal tissues including bone, cartilage and adipose.

Except for BM, MSCs were also found in almost all postnatal organs and tissues, including adipose, periosteum, synovial membrane, synovial fluid (SF), muscle, dermis, deciduous teeth, pericytes, trabecular bone, infrapatellar fat pad, articular cartilage, and umbilical cord blood (Bianco et al., 2008; Rebelatto et al., 2008). There is evidence that MSCs have been successfully isolated from nearly every tissue attempted so far. Although MSCs derived from various tissues present similar basic biological features, substantial disparities among them also exist, including (1) the difference in the expansion potential under identical culture conditions (Kern et al., 2006); (2) the difference in age-related functional properties. Based on these, it is necessary to determine the most appropriate MSC source for future clinical applications. To date, BM, adipose tissue, umbilical cord blood (UCB), and umbilical cord (UC) have usually been considered as the main sources of MSCs for tissue regeneration and engineering.

As the first choice for autologous transplantation with no ethical concerns, BM-derived MSCs appears to be irreplaceable (D'Ippolito et al., 2004; Kassem et al., 2004). However, aspirating BM from the patient is a highly invasive procedure and harvests only low numbers of cells (about 1–10 of 1×10^5 nucleated cells) (Gronthos et al., 2003). In addition, while increasing age leads to declining differentiation capacity and the maximal life span of MSCs from BM, their therapeutic potential might also diminish (Stenderup et al., 2003). Therefore, it is necessary to search for alternative sources of MSCs, especially in some refractory and complicated cases.

To date, more and more evidence has indicated that adipose tissue might provide an abundant alternative source of MSCs for autologous stem cell therapy, because adipose tissue is ubiquitous and easily obtainable in large quantities with little discomfort for patient (Mizuno, 2009; Zuk et al., 2002). Moreover, the expansion, proliferation and differentiation potential of adipose-derived MSCs are equivalent to BM-derived MSCs (Mizuno, 2009).

It usually takes two to three weeks to harvest enough MSCs in culture, which will bring barriers to conduct MSC therapies in the acute phase of some tissue injuries and diseases. Therefore, it is advisable to select appropriate allogeneic MSCs and establish a relevant cell repository in order to provide MSCs in a timely and convenient way.

Umbilical cord blood has been considered as an alternative source of allogeneic MSCs because it could be easily obtained and faced with few ethical problems, containing the youngest MSCs featuring the highest potential and low immunogenicity (Wagner et al., 2005; Bieback et al., 2004; Markov et al., 2007). However, UCB-derived MSCs are particularly rare (about 4 of 1×10^9 nucleated cells) and the rate of successful MSC isolation is low (<30%), especially from the UCB of full-term deliveries, which has posed a significant challenge to application.

In recent years, human umbilical cord has attracted increasing attention (Mageed et al., 2007). Compared with BM-derived MSCs, UC-derived MSCs are more efficient in terms of the expansion, proliferation and differentiation potential. Thus, it is generally accepted that the UC-derived MSCs could serve as an excellent allogeneic MSC source.

In addition, accumulating evidence has indicated that amniotic membrane is also an attractive source of MSC (Alviano et al., 2007). MSCs can be obtained from amniotic membrane which is supposed to be discarded, going through no intrusive procedures and posing no ethical conflict. Amniotic membrane-derived MSCs boast low immunogenicity, multi-potent differentiation ability and anti-

Table 2
The minimal requirements for MSCs suggested by ISCT.

Positive (+)	Negative (−)
CD73 (SH3, SH4)	CD14
CD90 (Thy-1)	CD19
CD105 (endoglin/SH2)	CD31
	CD34
	CD45
	HLA-DR

inflammatory function, similar to BM-MSCs (Chang et al., 2010; Kim et al., 2007). Actually, they have exhibited their therapeutic potential for multiple central nervous system (CNS) disorders (Chang et al., 2010; Sakuragawa et al., 2004) and vascular diseases (Alviano et al., 2007), etc.

2.2. Immune phenotype of MSCs

Up to now, intensive researches into the immune phenotypes of MSCs have been carried out, but the lack of unique specific markers for MSCs still presents several challenges for researchers: (1) Although series of phenotypic markers are identified to be expressed on MSCs (Table 1), there are still no unique specific markers that could be used to ensure homogeneity of MSCs. Therefore, the International Society for Cell Therapy (ISCT) has come up with the minimal set of standard criteria to identify MSCs (Dominici et al., 2006): (i) plastic adherent ability; (ii) expression of CD73, CD90, and CD105 and lack the expression of CD14, CD19, CD31, CD34, CD45, and HLA-DR surface molecules (Table 2); (iii) tripotential mesodermal differentiation capability into osteoblasts, chondrocytes, and adipocytes and (iv) immunomodulatory functions. (2) There are no established consensus markers that could reliably identify MSCs *in vivo*. Most researches attempted to search for MSCs *in vivo* through using the markers expressed by MSCs cultured *in vitro*. However, because these markers might be spuriously determined by the culture conditions rather than characteristic of MSCs *in situ*, it is difficult to identify, track or evaluate the phenotypic characteristics of MSCs *in vivo*. (3) Recent studies have shown that MSCs could be enriched through specific markers in specific tissue. For example, they are enriched from peripheral and UC blood through selecting CD133, or from BM through selecting stage-specific embryonic antigen (SSEA)-1, SSEA-4 (Tondreau et al., 2005; Anjos-Afonso and Bonnet, 2007; Gang et al., 2007). However, it should not be ignored that there is no standard method to isolate MSCs, and the difference in current isolation approaches might affect the comparability between relative experimental results. Moreover, some isolation schemes might introduce epigenetic and genetic changes in MSCs, which might also affect their plasticity and further therapeutic utility.

2.3. Differentiation potential of MSCs

In the past decades, the differentiation potential of MSCs has attracted much attention. Since the 1990s, increasing number of experimental data has demonstrated that MSCs could differentiate into mesodermal lineage as bone, cartilage, adipocyte, and connective stromal cell (Pittenger et al., 1999). Moreover, it also has been suggested that MSCs might also differentiate into not only ectodermal lineage as neuron, epithelialium, but also endodermal lineage as muscle and hepatocyte (Jiang et al., 2002; Lee et al., 2004; Tomita et al., 2007). Although most of these results came from *in vitro* experiments, they provide exciting evidence to recognize the differentiation of MSCs *in vivo*.

Regulated by the subtle microenvironment of local tissue, the differentiation of engrafted MSCs *in vivo* might be more complex. Based on current knowledge, it seems that, induced by the series of signals of local tissue, engrafted MSCs might differentiate into at least three types of cells: (1) Tissue-specific cells, which are required by injured tissues. For example, engrafted MSCs could differentiate into cardiomyocytes, smooth muscle cells, and vascular endothelial cells, which are important components of cardiac tissue (Gojo et al., 2003; Barbash et al., 2003; Psaltis et al., 2008). (2) Function-relative cells, which are desired by local tissue. This type of differentiated cells possibly participates in composing the special microenvironment or niche for tissue repair (Petrie Aronin and Tuan, 2010). (3) Regulatory cells, which contribute to tissue repair and regeneration through secretion of cytokines that might posses trophic and immunomodulatory functions (Ankrum and Karp, 2010).

The molecular and environmental mechanisms that control MSC differentiation are incompletely understood, and no unique phenotype marker has yet been identified to be associated with predictable differentiation potential of MSCs. Some hypotheses have been proposed to explain the mechanism of the differentiation potential of MSCs. For instance, Dennis et al. (1999) suggested that, in MSCs, there are some storage genes that could express and adjust their differentiation into varies lineages of cells under different conditions. Phinney and Prockop (2007) proposed that MSCs are equipped with motor proteins and a proteolytic arsenal that enables them to interact with and respond to signals from the extracellular matrix, and differentiate into unique structures such as muscle, bone, cartilage, or other connective tissues.

2.4. Migration and homing potential of MSCs

Numerous *in vivo* studies have shown that MSCs could migrate to injured, inflamed tissues from blood and exert therapeutic effects (Chapel et al., 2003; Chavakis et al., 2008). The detailed mechanism and efficiency of MSC migration might involve: (1) Specific receptors or ligands upregulated by injury tissues not only facilitate trafficking, adhesion, and infiltration of MSCs, but also provide MSCs with a specialized microenvironment or niche to support their self-renewal and maintain their multi-potentiality (Chapel et al., 2003). (2) Integrins, selectins, and chemokine receptors (Brooke et al., 2008) expressed on MSCs are involved in migration of MSCs across the endothelium. (3) MSCs are passively arrested in capillaries or microvessels including arterioles and post-capillary venules, and then directly interact with accessory cells and the release a wide array of soluble growth factors and trophic cytokines (Cselenyak et al., 2010; Le Blanc and Ringden, 2007).

2.5. Immunomodulatory properties of MSCs

The immunosuppressive and anti-inflammatory effects of MSCs were described in recent years. Although the underlying mechanisms responsible for these functions are not completely clarified so far, the following pathways might be involved: (1) MSC constitutively express low levels of major histocompatibility complex-I (MHC-I) molecules on their cell surface, and do not express MHC-II molecules and costimulatory molecules including B7-1 (CD80), B7-2 (CD86), or CD40 (Le Blanc and Ringden, 2007). Consequently, MSCs will not activate allogeneic or xenogeneic lymphocytes and lack immunogenicity. These characteristics support the possibility of exploiting universal donor MSCs for therapeutic applications, such as UC-derived MSCs. (2) MSCs were demonstrated to be able to suppress the activation and proliferation of both T and B lymphocytes partly by arresting these cells in the G0/G1 phase of the cell cycles (Jones et al., 2007; Corcione et al., 2006). As well as, MSCs might also interfere with the differentiation, maturation and function of dendritic cells through the mediation of soluble factors such as IL-6 and M-CSF (Djouad et al., 2007). (3) MSCs could modify the microenvironment of injured tissues, and protect damaged tissues

Table 3
Current status of clinical trials of MSCs.

Targeted disease	Clinical phase [Registered trial number (enrolled patient number)]					
	Undefined	I	I/II	II	II/III	III
Cardiovascular diseases						
Myocardial ischemia			2(50)	1(60)		
Dilated cardiomyopathy				2(80)		
Heart failure		1(48)	2(120)	3(200)		
Myocardial infarction		1(48)	2(45)	1(220)		
Neurological disorders						
Spinal cord injury		1(10)	1(80)[c]			
Stroke			1(78)	1(30)		
MSA				1(−)		
ALS		1(1)	1(24)			
Multiple sclerosis		1(24)	3(60)			
PD	1(5)					
Neuroblastoma	1(15)					
Pancreatic disorders						
Type I diabetes	1(20)	1(24)	1(30)	1(60)	1(80)	
Type II diabetes		1(24)	1(100)			
Liver diseases						
Hepatic cirrhosis	2(13)		1(30)	3(160)		
Liver failure			1(92)			
Hypercholesterolemia		1(1)[c]				
Gastrointestinal diseases						
Crohn's disease			2(36)	1(10)[c]		1(200)[c]
						1(270)
Kidney diseases						
Kidney injury			1(15)			
Kidney transplant	1(60)		3(41)			
Lupus nephritis			1(20)			
Kidney tubular necrosis		1(15)				
Lung diseases						
COPD			1(60)			
Bone/Cartilage defects						
Articula cartilage defects	1(50)	2(56)			1(25)	
Tibial fracture	1(186)		1(24)			
Multiple trauma	1(90)					
Osteonecrosis of the femoral head	2(51)					
Osteogenesis imperfecta	1(14)[c]	1(9)[c]				
		1(12)				
OA	1(30)					1(104)
Osteodysplasia		1(8)[c]				
Bone neoplasms					1(50)	
Bone fusion	1(100)	6(454)	2(124)			
Meniscectomy		1(60)				
Periodontal tissue			1(9)[c]			
Limb ischemia						
Dabetic foot					1(80)	
Critical limb ischemia		2(56)		1(30)[c]		
Skin diseases						
Diabetic wound	1(250)					
Epidermolysis bullosa				1(75)		
Autoimmune diseases						
Systemic sclerosis			1(20)			
pSS			1(20)			
SLE			1(20)			
Hematopathy						
GVHD		1(49)	5(80)	7(452)		1(240)[c]
				1(33)[c]		
MDS				1(30)		

These data were searched on 24 July, 2010 from the website of ClinicalTrials.gov (http://www.clinicaltrials.gov). The following keywords including "mesenchymal stem cells", "mesenchymal stromal cells", "multi-potent stromal cells", "multi-potent progenitor cells", "bone marrow stromal cells", "stem cells for spinal fusion", and "connective tissue progenitor" were used.
MSA: Multiple system atrophy; ALS: Amyotrophic lateral sclerosis; PD: Parkinson's disease; COPD: Chronic obstructive pulmonary disease; OA: Osteoarthritis; pSS: Sjogren's syndrome; SLE: Systemic lupus erythematosus; GVHD: Graft-versus-host disease; MDS: Myelodysplastic syndromes. [c] represents completed clinical trial.

through releasing anti-inflammatory and anti-apoptotic molecules (Le Blanc and Ringden, 2007; Meirelles Lda et al., 2009).

Due to the immunomodulatory properties possessed by MSCs, MSC transplanation has been used for the treatment of GVHD implicated in allogeneic stem cell transplantation, and several autoimmune diseases, including autoimmune type 1 diabetes (Fiorina et al., 2009), rheumatoid arthritis (RA) (Bouffi et al., 2009), systemic lupus erythematosus (SLE) (Zhang et al., 2010) and multi-

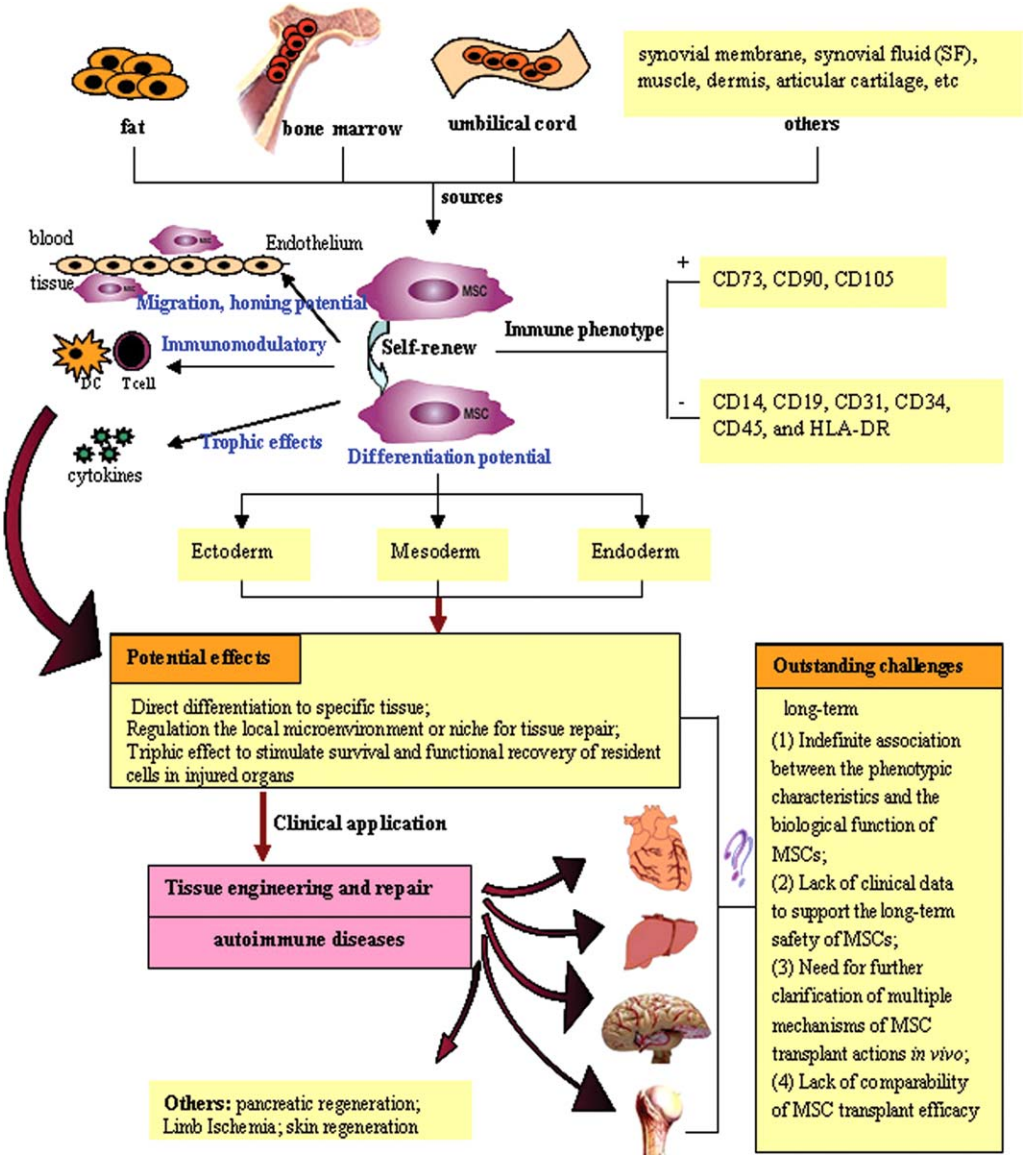

Fig. 1. The biological characteristics and clinical applications of MSCs. MSCs have been shown to exert their therapeutic function in various disorders characterized by tissue injury through various mechanisms, including the potential of differentiation, migration, homing to injured tissue, as well as immunomodulatory and trophic effect. However, several outstanding challenges involved in therapeutic application of MSCs remain, such as (1) indefinite association between the phenotypic characteristics and the biological functions of MSCs; (2) lack of clinical data to support the long-term safety of MSCs; (3) need for further clarification of multiple mechanisms of MSC transplant actions *in vivo*; and (4) lack of comparability of MSC transplant efficacy.

ple sclerosis (MS) (Tyndall A and EULAR Stromal Cell Translational Group, 2010; Martino et al., 2010).

2.6. Trophic effect of MSCs

Recently, it is observed that the trophic effect of MSCs is of great significance in tissue regeneration. After engraftment, MSCs could contribute to tissue repair by secreting a number of trophic molecules which include soluble extracellular matrix glycoproteins, cytokines, and growth factors (Ankrum and Karp, 2010), and direct cell-to-cell (MSCs and adjacent tissue cells) connection (Plotnikov et al., 2008). It has been verified that these trophic molecules could not only reduce inflammation, apoptosis and fibrosis of damaged tissues, but also stimulate tissue cell regeneration. Although there is evidence showed that MSCs and certain tissue cells such as cardiomyocytes can interact with each other via small diameter nanotubes, and exchange membrane and organelle parts such as mitochondria or other cytoplasmatic components, the underlying mechanism of cell-to-cell connection and their possible roles during tissue regeneration remains to be further investigated (Cselenyak et al., 2010; Plotnikov et al., 2008).

In the acute phase of injury, while MSC differentiation is unavailable, MSCs seem to play its role in regeneration mainly through their trophic function (van Poll et al., 2008). Nowadays, the trophic effect of MSCs has been reported in various disease models, including radiation burn injuries, myocardial infarction (MI), and Parkinson's disease, etc. It might give rise to a shift from utilizing differentiation properties of MSC for therapy to taking advantage of trophic properties of MSC, especially during the acute phase of injuries or diseases.

3. Clinical application of MSCs in tissue regeneration and disease therapy

The therapeutic potential of MSCs has already been recognized, which might be the most exciting advance in cell therapy

after the widespread use of HSCT. The immunomodulatory properties of MSCs are currently being exploited not only to improve haematopoietic microenvironment reconstitution and prevent GVHD after allogeneic HSCT, but also to treat autoimmune diseases. In addition, the proliferation, and differentiation potential of MSCs could be also widely utilized for clinical application in tissue engineering and repair (Fig. 1).

Currently, the effective therapeutic benefits of MSC transplantation have been supported by increasing numbers of clinical trials on various disorders. As summarized in Table 3, a total number of 108 registered clinical trials involving 42 different diseases can be found in the website of ClinicalTrials.gov (http://www.clinicaltrials.gov). However, several potential questions and the need for an improved understanding of the mechanism involved in therapeutic application of MSCs remain. Thus, more work should be done before MSC transplantation becomes a routine approach in the clinic.

3.1. MSCs in ischemic heart diseases

As a candidate for cardiac therapy, MSCs have provided comprehensive functional benefits, which include diminishing myocardial scars and infarct size, restoring myocardial mechano-energetics, improving regional and global ventricular function, and increasing vascular density and myocardial perfusion (Psaltis et al., 2008; Ohnishi et al., 2007), in myocardial infarction and ischemic cardiomyopathy. Also, there is evidence demonstrating MSC benefits in models of dilated cardiomyopathy, arrhythmia (Chin et al., 2010; Martínez de Ilárduya et al., 2009).

MSCs could make therapeutic contribution to myocardial repair by virtue of multiple factors including: (1) directly differentiating into cardiac tissue including cardiomyocytes, smooth muscle cells, and vascular endothelial cells (Gojo et al., 2003; Barbash et al., 2003); (2) secreting a variety of cytokines and growth factors that have trophic effects (Kinnaird et al., 2004; Caplan and Dennis, 2006); (3) attenuating the severe inflammation of the injured myocardial tissue through local immunosuppressive functions (Du et al., 2008; Guo et al., 2007); (4) stimulating endogenous repair (Paul et al., 2009; Nakanishi et al., 2008).

However, it could not be denied that several questions still need to be answered. (1) The efficiency of MSC differentiation into functional cardiomyocytes *in vivo* has not been defined. The cardiac and myocyte markers such as GATA4, Nkx2.5, a-sarcomeric actinin, phospholamban, and cTnT have been detected in the MSC-differentiated cells (Perán et al., 2010; Toma et al., 2002), which verified this differentiation does exist. However, most studies have suggested that it occurs rarely under physiological conditions. (2) The detailed mechanism responsible for homing of MSCs is far from clear, although chemokine stromal cell-derived factor-1 (SDF-1), granulocyte colony-stimulating factor (G-CSF), and the pericyte-specific markers CD146 and 3G5 (Cheng et al., 2008; Crisan et al., 2009; Khan et al., 2010) have been implicated in homing of MSCs to sites of ischemic myocardium. (3) The optimal engrafting timing and pathways for MSC transplantation remains unknown. Because diseased tissue environments often exhibit pathological levels of ischemia, inflammation, and fibrosis, which could impair cell survival, it is necessary to figure out appropriate timing and methods to prevent MSC apoptosis and improve their therapeutic potential in the ischemic heart. Many methods have been recently developed, such as genetic modification and preconditioning *in vitro* (Segers and Lee, 2008). (4) The long-term efficacy of MSC transplantation is not yet clear. In animal experiments, Dai et al. (2005) found that MSCs could survive in MI tissue for six months after transplantation, and express proteins characteristic of muscle and endothelial cells; however, its improvement of heart function seemed to be transient, being apparent after four weeks but fading away after six months. (5) The long-term safety of MSC transplantation remains a critical and unresolved concern. For example, the prevalence and severity of complications such as abnormal tissue development and arrhythmia are not clear.

Based on these questions mentioned above, rigorous investigation should be carried out to search for evidence of efficiency and safety of MSCs *in vivo*. This will make it necessary to conduct sufficient follow-up after MSC transplantation, combining with sensitive and accurate tissue analysis through myocardial imaging, histopathology, and comprehensive electrophysiologic evaluation.

3.2. MSCs in pancreatic regeneration

Recent studies have demonstrated that MSC transplantation exerts a protective effect on diabetes mellitus. Studies on diabetes model have shown that BM-derived MSCs could localize to the pancreas after intravenous transplantation, and substantially lower the level of blood sugar (Ezquer et al., 2008). Similar results were observed in studies on MSCs isolated from the Wharton's jelly of the umbilical cord (HUMSCs), which successfully differentiated into mature islet-like cell clusters and possessed insulin-producing ability *in vitro* and *in vivo* (Chao et al., 2008). However, the mechanisms of the therapeutic effects are undefined. Transplanted MSCs are thought to lower the blood sugar through generating new β-cells, which involves at least two mechanisms: (1) secretion of trophic cytokines to promote endogenous pancreatic stem cells in the ductal epithelium differentiate into new β-cells; (2) direct differentiation into functionally competent, new β-cells *in vivo* (Xie et al., 2009). Furthermore, the present studies also have shown that the mechanisms mentioned above might not be the only explanation for the therapeutic efficacy of MSCs in diabetes treatment, because MSCs naturally produce a variety of cytokines and growth factors which could promote the survival of surrounding cells, and improve the microenvironment of pancreas (Lee et al., 2006; Park et al., 2010).

3.3. MSCs in neurological disorders

In recent years, MSCs have been considered as a promising therapeutic strategy for acute injury and progressive degenerative diseases of the central nervous system, such as spinal cord injury (Himes et al., 2006), stroke, Parkinson's Disease (PD) (Park et al., 2008), autoimmune encephalomyelitis (EAE) (Zhang et al., 2006), amyotrophic lateral sclerosis (Choi et al., 2010), and multiple system atrophy (MSA) (Lee and Park, 2009). According to recent studies, the neuroprotective effect of MSCs is mediated by at least two major mechanisms: (1) production of various trophic factors, including brain-derived neurotrophic factors (BDNF) (Wilkins et al., 2009), nerve growth factor (NGF) (Cho et al., 2010), and insulin-like growth factor-1 (IGF-1) (Wakabayashi et al., 2010), that contribute to recovering neurobehavioral function and stimulating endogenous regeneration; (2) exerting immunoregulatory properties through homing to damaged brain tissues, thereby resulting in reduction of apoptosis and improvement neuronal cell survival.

Despite the above-mentioned studies have reported exciting results, it is not yet clear whether MSCs could differentiate into neural cells *in vivo*. A recent study showed that before transplantation, cultured hMSCs *in vitro* expressed markers of both undifferentiated and committed neural cells, including nestin, GAP-43, NSE, β-tubulin III and MAP-2, but did not express glial or specific neuronal markers (Blandini et al., 2010). However, following transplantation, some hMSCs expressed a glial-like phenotype and lost the remarkable positivity for nestin that originally expressed *in vitro*. The mechanisms of this phenotypic shift have not yet been clarified.

Taken together, although the neuronal differentiation of MSCs is not yet clear, the significant neuroprotective effects of the grafted

MSCs make them strong candidates for the development of cell-based therapies for CNS injuries and diseases.

3.4. MSCs in hepatic cirrhosis

Generally, there is less investigation into the therapeutic potential of MSCs in liver-related diseases when compared with other fields. Nowadays, the underlying mechanism of therapeutic potential of MSCs on hepatic cirrhosis is complicated and far from clear. Moreover, the long-term fate of the engrafted MSCs also remains unclear. However, preclinical studies have provided evidence for *in vivo* hepatic differentiation of MSCs (Sato et al., 2005; Chamberlain et al., 2007), and also demonstrated that MSCs could attenuate the progression of hepatic fibrogenesis, through secreting molecules which possess anti-fibrogenic effects in injured liver (Wang et al., 2009). Based on knowledge from preclinical studies, Mohamadnejad et al. (2007) performed a small-scale clinical study in which four patients with decompensated liver cirrhosis received MSC transplantation. Improved liver functions and increased liver volumes were observed at the end of follow-up (after 12 months).

3.5. MSCs in limb ischemia

Previous studies (Al-Khaldi et al., 2003; Xu et al., 2010) have demonstrated that transplantation of MSCs induced angiogenesis accompanied by an increase of blood flow and capillary density in the ischemic limb. Taken together, MSCs could exert the angiogenic potential through multiple pathways, including: (1) differentiation into smooth muscle and vascular endothelial cell lineages, which contribute to remodeling vessels; (2) release of various angiogenic factors and stem/progenitor cell chemokines, including vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) (Quevedo et al., 2009), which stimulate survival and functional recovery of resident cells in injured organs through paracrine mechanisms and recruitment of local precursors. (3) paracrine effects on local vascular cells, leading to recruitment on circulating stem and/or progenitor cells.

Nevertheless, there are still some controversies over the mechanism of the angiogenic potential of MSCs. For example, some researches indicated that the actual number of MSCs differentiated into vascular structures was quite low (less than 1%). Thus, it is suggested that the angiogenic potential of MSCs might be mostly related to their trophic effects (Kinnaird et al., 2004) rather than their differentiation potential.

Although the efficiency of MSC treatment was repeatedly observed in animal models with limb ischemia (Al-Khaldi et al., 2003; Xu et al., 2010), therapies with purified MSCs in patients are still in the stage of clinical trial (see Table 3). Whereas, limited clinical data demonstrated that local autologous bone marrow stem cell (containing MSCs) transplantation can effectively improve leg perfusion in about 80% patients with critical limb ischemia, thus avoiding the consideration of amputation in these patients (Amann et al., 2009; Procházka et al., 2009). These reports suggest that MSC-based therapy for patients with limb ischemia may be efficient and safe.

3.6. MSCs in skin regeneration

Recently, significant progress has been made in the regeneration of the dermis in wound healing through transplantation of MSCs. Wu et al. (2007) have found that MSCs could significantly accelerate wound closure, re-epithelialization and angiogenesis. Notably, BM-MSCs engrafted into the wound could express the keratinocyte-specific protein and form glandular structures, which suggested their contribution to cutaneous regeneration (Wu et al., 2007).

Moreover, our research group (Sheng et al., 2009) presented the first report of successful transplantation of MSCs in regenerating functional sweat glands. We induced BM-MSCs to acquire the phenotype of sweat gland cells (SGCs) *in vitro* and transplanted these cells into fresh skin wounds, resulting from excision of anhydrotic scars after healing of deep burn injury, in five patients. In a 2–12 months follow-up after the procedure, there was recovery of perspiration function in all the MSC-transplanted areas, which indicates that MSC-transformed SGCs were involved in the recovery of functional sweat glands.

Although BM-MSCs have evoked significant interest owing to their potential for therapeutic applications in human skin tissues (Fu et al., 2006), several questions need to be addressed. For example, we know little about the appropriate environment or niche, which is a vital factor in the process of MSC differentiation. Therefore, it is important to identify the molecule or protein necessary for differentiation. Furthermore, the optimal number of cells needed for transplantation for different patients and the appropriate transplantation timing need to be determined.

3.7. MSCs in graft-versus-host disease

The application of MSCs to treat GVHD has generated enormous interest since the first successful report by Le Blanc et al. (2004) A multi-center, Phase II experimental study among 55 patients with steroid-refractory acute GVHD showed that more than half of the patients responded to MSC-based treatment and acquired higher overall survival 2 years (Le Blanc et al., 2008). However, in an attempt to characterize the underlying mechanisms, a number of studies have shown contradictory results. For example, in a model of haploidentical haematopoietic graft, lethal GVHD was abrogated in mice receiving MSCs, whereas MSCs had no effect on the severity of GVHD in another model (Yanez et al., 2006; Sudres et al., 2006). These contradictory results highlight the need to better determine the parameters of MSC transplantation, including the timing, frequency and dose.

3.8. MSCs in rheumatoid arthritis

Rheumatoid arthritis is believed to be a T-cell-driven inflammatory synovitis disease leading to joint destruction. Although the biological roles of MSCs in RA pathophysiology are still unknown, it has been shown that MSCs or MSC-differentiated chondrocytes were able to inhibit the proliferation and activation of collagen type II-stimulated T-cell in a dose-dependent manner (Zheng et al., 2008). Moreover, they also inhibited the secretion of the pro-inflammatory cytokines such as IFN-γ and TNF-α by $CD4^+$ and $CD8^+$ cells, while increasing the secretion of IL-10 and restoring the secretion of IL-4 (Chen and Tuan, 2008). Based on these studies, the anti-inflammatory and immune-suppressive properties of MSCs indicate that they might be effective candidate cells for cartilage and bone repair therapy in RA.

3.9. MSCs in osteoarthritis (OA) and other bone/cartilage defects

OA is a disease that affects mostly cartilage and bone, causing progressive and often severe inflammation. The extensively proliferative potential, chondrogenic differentiation, and anti-inflammatory function of MSCs make them a competitive option in this field.

Previously, MSCs have been used for cartilage tissue engineering in cartilage repair, via their chondrogenic differentiation after being loaded on a 3-D scaffold (Chen and Tuan, 2008). In addition, in phase I/II clinical trials, MSCs have also been used directly in cell therapy for OA cartilage repair *in situ*. However, the inefficient engraftment of MSCs presents a challenge in using MSCs systematically or locally

for arthritis prevention and treatment (Noth et al., 2008). Current studies indicate that MSC-based procedures mainly rely on their trophic effects and their anti-inflammatory and immunosuppressive abilities, which could significantly affect the local environment and the regenerative function of resident endogenous tissue progenitor cells.

Except for clinical applications in patients with OA, MSC transplantation was also proposed to be used for the repair of non-osteoarthritic cartilage defect, bone defect, and non-union, etc. (Jorgensen et al., 2004). When submitted to specific host environment, MSCs can differentiate into chondrocytes or osteoblasts and secrete cartilaginous matrix. However, perfect integration of the regenerated tissue within the surrounding host tissue may require the combined use of MSCs and biomaterial scaffolds.

4. The potential barriers that prevent MSC transplantation to become a routine therapeutic approach in the clinic

Although MSC researches over the past three decades have generated great excitement and promises, and several clinical trials are underway for a variety of disorders mentioned above, there are still several critical potential problems in therapeutic application of MSCs.

4.1. The indefinite association between the phenotypic characteristics and the biological function of MSCs

Current researches indicate that specific phenotypic markers expressed on MSCs might reflect their distinct functional properties. On basis of this, according to the functional attributes of MSCs, the phenotypic markers expressed on MSCs could be classified into at least three types: (1) Lineage-specific molecules, which offer MSCs multiple differentiation potential, and might determine the direction of their differentiation. (2) Series of molecules such as pericyte markers, P-selectin, integrins, and chemokine receptor, which contribute to the homing, migration, adhesion and chemokine-induced recruitment abilities of MSCs. (3) Immunomodulation-related molecules which provide MSCs with the immunosuppressive capacity to modulate immune and anti-inflammatory responses. However, the corresponding association between the phenotypic characteristics and the biological function of MSCs is not yet clearly understood (Ohishi and Schipani, 2010).

There is an increasing number of evidence that the different phenotypic shifts of MSCs could be found *in vitro* or *in vivo*, under different microenvironment conditions. However, the mechanisms that mediate the phenotypic shifts of MSCs remain unresolved, and more studies are required to substantiate them. This situation presents challenges to basic and clinic researches of MSCs, and also highlights the need for a unified standard to evaluate the phenotypic characteristics and relevant functions of MSCs.

4.2. Lacking clinical data to support the long-term safety of MSCs

MSC transplantation is currently considered to be relatively safe, but its long-term safety still remains unclear. Large-scale controlled and double-blinded clinical trials are required to assess the safety of MSCs before this cell transplantation becomes a routine therapeutic approach. Moreover, prior to MSC transplantation, objectively standardized evaluation criterion to assess the safety and efficiency of the therapeutic procedures of MSC transplantation must be established, such as rigorous quality control (QC) standards for the production of GMP-grade MSCs, and objective evaluation criterion for the phenotype, functional potential, and microbiological safety of MSCs.

4.3. Multiple mechanisms of MSC transplant actions in vivo should be further determined

Although MSC transplantation has shown exciting therapeutic potential in various disorders, the mechanisms of MSC transplants remain unclear, and some of the results that came from current preclinical experiments and clinical trials are controversial. Critical questions pertinent to the mechanism of MSCs include the following.

(1) What is the fundamental mechanism of MSC action *in vivo*? Based on the knowledge from existing studies, MSC functions include: (i) multi-lineage differentiation of MSCs which contributes to reconstruction of injured tissue; (ii) immunemodulatory capacity; and (iii) trophic effects. However, much of our knowledge on MSCs is based on *in vitro* experiments, and there are insufficient researches to figure out the physiological role of these cells *in vivo*. For instance, numerous data have indicated that MSCs could differentiate into multi-lineage cell types *in vitro*. Nevertheless, there is not enough direct evidence for the detailed differentiation route of MSCs *in vivo*. Furthermore, it is unknown whether MSCs could exert different dominant functions in different phases of diseases, and it remains unknown how to balance or shift these functions appropriately.
(2) How long could engrafted MSCs survive *in vivo* and maintain their functions? The data deriving from MSC labeling and imaging studies indicates that engrafted MSCs could migrate into injured tissue and survive there for at least a few months, benefiting the repair of injured tissue. However, the hostile microenvironment of injured tissue, including the ischemia, excessive inflammation, and fibrosis, might prevent the recruitment and survival of exogenous MSCs. Various studies have indicated that the extensive apoptosis or death of transplanted MSCs occur within the first week. Consequently, this will reduce the success rate of MSC therapies and present a formidable barrier to MSC transplantation. The detailed mechanisms of the interaction of MSCs with the intrinsic microenvironment are far more complex than previously thought, while the limited types of experimental animals, few clinical trials and insufficient information of long-term MSC engraftment have not provided enough evidence to determine these mechanisms. Accordingly, what are the optimal dose, timing, frequency, and routes of MSC transplantation in different phases of injuries and diseases? These important clinical questions need to be addressed urgently.

4.4. Lack of comparability of MSC transplant efficacy among clinical trials

Current preclinical and clinical trials sometimes could not exhibit stable reproducible therapeutic efficacy of MSCs in disease models or patients. This might be due to a number of factors, including the lack of universally accepted criterion to define the MSC phenotype and functional properties. In addition, because the lack homogeneity and comparability among trials will bring difficulties to the clinical application of MSCs, current basic and clinical research data of MSCs often comes from scattered, small-sample studies. In order to cope with these challenges, large-scale, multi-center clinical trials are required before MSC transplantation becomes a regular therapy. Moreover, there are many problems need to be answered, such as how to stratify and select the most responsive patients and what is the optimal dosing regimen.

Based on the above-mentioned analyses, the numbers and phases of current status of MSC clinical trials as presented in Table 3, we proposed that MSC-based therapy might be most promising for cardiovascular diseases and neurological disorders. In contrast, in

view of the potential promotion roles of MSCs for tumor cell survival and metastasis by their mutual interactions (Karnoub et al., 2007; Roorda et al., 2009), we should be extremely cautious before infusing MSCs into the patients with tumors such as neuroblastoma and bone neoplasms, and patients with the risk of tumor transformation like myelodysplastic syndromes (MDS).

5. Conclusions and further perspectives

Considering the clinical and experimental data together, MSC-based therapies in various diseases have generated great excitement. However, recent researches have also highlighted that it is not easy to translate the potential of MSC therapy into actual practice and many of the barriers mentioned above need to be overcome before this therapy is widely used.

Therefore, it is particularly important to implement multicenter and large-scale clinical trials designed to investigate the safety and efficacy of MSC transplantation. The MSC-based treatments are also need to be assessed by a ministry-approved regulator, which is authorized to carry out rigorous monitoring required by clinical trial protocols.

These interventions might make MSC transplantation a routine therapy in the clinic, and avoid unproven treatment which will bring risks to patients' health and potentially damage the reputation of stem cell research.

In conclusion, although more work need to be done, MSC-based therapies have significant potential to reduce mortality, and improve quality of life in patients with severe disease.

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