Cell Transplantation, Vol. 23, pp. 513–529, 2014
Printed in the USA.

0963-6897/14 $90.00 + .00
DOI: http://dx.doi.org/10.3727/096368914X678436
E-ISSN 1555-3892
www.cognizantcommunication.com

Review

Mesenchymal Stem Cell Insights: Prospects in Cardiovascular Therapy

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Ischemic heart damage usually triggers cardiomyopathological remodeling and fibrosis, thus promoting the development of heart functional failure. Mesenchymal stem cells (MSCs) are a heterogeneous group of cells in culture, with multipotent and hypoimmunogenic characters to aid tissue repair and avoid immune responses, respectively. Numerous experimental findings have proven the feasibility, safety, and efficiency of MSC therapy for cardiac regeneration. Despite that the exact mechanism remains unclear, the therapeutic ability of MSCs to treat ischemia heart diseases has been tested in phase I/II clinical trials. Based on encouraging preliminary findings, MSCs might become a potentially efficacious tool in the therapeutic options available to treat ischemic and nonischemic cardiovascular disorders. The molecular mechanism behind the efficacy of MSCs on promoting engraftment and accelerating the speed of heart functional recovery is still waiting for clarification. It is hypothesized that cardiomyocyte regeneration, paracrine mechanisms for cardiac repair, optimization of the niche for cell survival, and cardiac remodeling by inflammatory control are involved in the interaction between MSCs and the damaged myocardial environment. This review focuses on recent experimental and clinical findings related to cellular cardiomyoplasticity. We focus on MSCs, highlighting their roles in cardiac tissue repair, transdifferentiation, the MSC niche in myocardial tissues, discuss their therapeutic efficacy that has been tested for cardiac therapy, and the current bottleneck of MSC-based cardiac therapies.

Key words: Mesenchymal stem cells (MSCs); Myocardial infarction; Cellular therapy; Differentiation; Regeneration; Niche; Paracrine

INTRODUCTION

Ischemic heart disease (IHD), also known as coronary artery disease (CAD), is the most common type of heart disease and cause of heart attacks. IHD is the leading cause of death worldwide (34). The myocardium has a high demand for oxygen supply. Interruption of the blood supply for a certain period followed by reperfusion always causes irreversible myocardial damage, described as ischemia/reperfusion (IR) injury. IR-induced heart tissue damage usually triggers a common cardiomyopathy of pathological remodeling and fibrosis, promoting the development of functional heart failure. IR-induced myocardial infarction (MI) can lead to heart failure, which is fatal within 5 years for 65% of the patients. Effective cardioprotection against IR is one of the most important goals of experimental and clinical research in cardiology.

Received October 30, 2013; final acceptance February 6, 2014.
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According to the traditional concept, the heart only has negligible intrinsic regenerative capacity, and therefore, recovery of the cardiac tissue in the infarct region could enhance the functional activity of the heart. However, the presence of residential cardiac stem cells was first reported in 2003 (10). Moreover, several experimental findings have shown strong evidence that cardiomyocytes may be able to undergo mitosis and initiate a limited regeneration through recruitment of residential or circulating stem cells in the damaged site of the heart (11,15,42,45,50). This new finding not only challenged our understanding of the self-renewing characteristics of the heart but also opened a new therapeutic option for cardiovascular diseases.

Stem cells have been widely used to regenerate various damaged tissues in animal models. The potential for using differentiated human embryonic stem cells (ESCs) to treat degenerative diseases and injuries has long been evident. However, the ethical issues and the risk of forming a teratoma have prompted huge efforts to find alternative approaches that could use adult stem cells or progenitor cells instead. Adult somatic stem cells have been recently identified in multiple organs, and attempts were made to utilize them for therapeutic regeneration of cardiovascular diseases (28). Mesenchymal stem cells (MSCs) are a rare but unique adult stem cell population with self-renewal and differentiation abilities and are able to replenish a variety of specific cell types. MSCs could be used from an autologous origin or may be used from an allogeneic origin due to their proposed hypoimmunogenic character in cell transplantation (6,14). MSCs could be used directly without predifferentiation in culture, thus avoiding exposure to animal-derived reagents and preventing the development of chromosomal abnormalities. Alternatively, MSCs can be expanded and stored for usage whenever necessary. These advantages have led to the rapid testing for the clinical applications of MSCs.

According to in vitro studies of cardiomyocyte differentiation and in vivo animal experiments on cardiac functional improvement, MSCs can ameliorate cardiac function after MI (68,114). Several human clinical trial results have shown that MSC-based cell therapy possesses remarkable clinical efficacy in functional improvements including ventricular volumes, infarction size, ejection fraction, and myocardial perfusion (19,41,71). Many clinical trials targeting acute and chronic ischemic heart failure are still ongoing. The mechanism(s) of action of MSCs on myocardial regeneration may arise from their direct transdifferentiation into cardiac cell lineages, paracrine actions via cytokine secretion, optimization of the niche for residential cardiac stem cells, and inflammatory control. Despite the exciting possibilities that MSC-based cell therapy could have major beneficial effects on myocyte regeneration, a lack of standardization in the cell processing and delivery methods has resulted in inconsistent outcomes, poor engraftment, and short survival times in human trials (65,100,101,111).

Here we review the status of research on cell transplantation in experimental animals and humans of MI. We focus on MSCs, highlighting their roles in tissue repair and inflammatory regulation; maintenance of the MSC-niche in myocardial tissues; their therapeutic efficacy, which is currently being tested for cardiac therapy; and the current bottleneck over the successful use of MSC-based techniques on cardiac therapy.

CELLULAR THERAPY FOR CARDIOVASCULAR DISEASES

Cellular cardiomyoplasty is a new therapeutic approach characterized by the delivery of appropriate healthy donor cells to the injured myocardium to replace the damaged cardiomyocytes. The therapeutic efficacy of cellular therapy for heart disease is highly determined by the myocardiopathological process, including myocardial ischemia, cardiac dysfunction, or a combination of both (46). In the presence of IR, cellular transplantation is most likely to be effective if it can contribute two capabilities: [1] It provides a renewable source of functional cardiomyocytes and [2] it can contribute to the development of blood vessels to support and nourish newly formed cardiomyocytes and the surrounding ischemic myocardium. In the past decade, various somatic mature cell types have been used to repair infarcted myocardium, including adult cardiomyocytes, skeletal myoblasts, immortalized myoblasts, smooth muscle cells, and fibroblasts (44,59,89,96). Skeletal myoblasts are a group of satellite cells found beneath the basal membrane of muscle fibers and can be isolated from skeletal muscle biopsies and expanded in vitro. Even though skeletal myoblasts cannot differentiate into functional cardiomyocytes with electromechanical coupling, injection of skeletal myoblasts into the infarcted area did improve the regional and global left ventricular ejection fraction (LVEF) (38,73,74). However, considerations for the modified product by cell enhancement techniques have been formulated to generate a second generation of skeletal myoblasts. The therapeutic efficacy of somatic mature cells is limited and transitioned. Therefore, various types of stem/progenitor cells have been widely used to regenerate cardiac tissues damaged by myocardial infarction (4,10,33,83,103) (Table 1).

Stem or progenitor cells are usually classified according to the following criteria: origin, cell-derived organ or tissue type, surface markers, and final differentiation fate. Stem cells are primitive, undifferentiated, multipotent cells, which retain the ability to self-renew and possess multilineage differentiation. They can be obtained from the embryo, fetus, and various parts of the adult body.

Table 1. Major Cell Types for Cardiac Cell Therapy

Type	Source	Advantages	Disadvantages	Ref.
Stem cell				
ESCs	Inner cell mass	Totipotent and highly expandable	Immunosuppression required, ethical debate, and tumorgenesis	27,33,56,58
iPSCs	Fibroblast (by reprogramming)	Pluripotent and ES-like	Tumorgenesis	86,109,110,123
HSCs	Bone marrow/peripheral blood	Multipotent, autologous transplantation, and different lineage of cells	Few cell number	129
MSCs	Bone marrow/adipose tissue	Allogenic/autologous transplantation, lack of immunogenicity, pluripotent	Expansion if required	49,99,128
Progenitor				
EPCs	Bone marrow/peripheral	Autologous transplantation, monopotent	Need for expansion	48,55,133
Fetal cardiomyocytes	Fetal heart	Multipotent and cardiomyocyte phenotype	Immunosuppression required, ethical debate, short survival, and limited supply	10,83
UCBCs	Umbilical vein	Multipotent, allogenic/ autologous transplantation, low immunogenicity	Few cell number, requires expansion	29
Mature cell				
Skeletal myoblasts	Skeletal muscle	Autologous transplantation and high yield	Electrophysiologically incompatible, lack of gap junction	73,74
Adult cardiomyocytes	Heart	Multipotent and cardiomyocyte phenotype	Immunosuppression required, short survival, and limited supply	10,83

ESCs, embryonic stem cells; iPSCs, induced pluripotent stem cells; HSCs, hematopoietic stem cells; MSCs, mesenchymal stem cells; EPCs, endothelial progenitor cells; UCBCs, umbilical cord blood cells.

ESCs from mice, primates, and humans can differentiate into all three germ layers (112). The potential for using differentiated human ESCs to treat degenerative disease and injuries has long been evident. ESCs can be differentiated into beating cardiac myocytes and electromechanically coupled to the host cardiac cells (27,56). Moreover, ESC-derived cardiac myocytes most resemble embryonic cardiac myocytes expressing the same cardiac-restricted transcription factors such as guanine–adenine–thymine–adenine-binding protein 4 (GATA4), NK2 homeobox 5 (Nkx2.5), and myocyte enhancer factor 2C (MEF2C) (33). In addition, transplantation of ESC-derived cardiac myocytes into a rodent MI model has shown successful engraftment and cardiac functional improvement (18,58,60,62,72,117). However, ethical concerns, teratoma formation, and immune rejection limit their usage. The recent discovery of inducible pluripotent stem cells (iPSCs) which are ESC-like cells and are generated by reprogramming adult fibroblasts with ESC pluripotency regulating genes, may help resolve the ethical and immunogenic issues associated with the use of ESCs (86,109,110,123).

Adult stem cells are defined as undifferentiated and multipotent cells from an individual after birth. To our knowledge, the regeneration capacity of the mammalian heart is limited. Recently, a population of resident cardiac stem cells (CSCs) with the potential to differentiate into cardiomyocytes was discovered in a specific niche of adult mammalian myocardium tissue (10,63,83,125). This finding is a milestone in cardiovascular biology. CSCs become activated and proliferate following tissue damage. CSCs can replace the apoptotic cells and maintain cardiomyocyte turnover. At present, it is unclear if CSCs are of a distinct type or whether they represent different stages of a single cell lineage. In addition, the limited supply, short survival time, and the immunosuppression required are limiting the clinical use of CSCs. To this end, well-characterized bone marrow-derived hematopoietic stem cells (HSCs) have been shown to differentiate into cardiomyocytes in culture, making them of particular

interest in the treatment of cardiac diseases (129). Direct injection of bone marrow-derived c-kit$^+$Lin$^-$ cells into the infarct region or the mobilization of cells from endogenous reservoirs have led to significant improvement in cardiac function (87,88).

Mature tissue somatic cells have been shown to contain organ-specific progenitors or mature cells. Endothelial progenitor cells (EPCs) present a subset of HSCs with cluster of differentiation 34 (CD34) HSC and fetal liver kinase 1 (Flk-1) endothelial markers. Results from animal experiments indicated that injection of EPCs into infarcted myocardium improved LVEF and inhibited fibrosis (48,55). Moreover, preclinical trials indicated that EPCs are involved in vessel formation and vascular homeostasis after IR (122,133). However, a shortage in their supply means that there is a need for expansion, which limits the clinical use of EPCs. There is also some controversy over which cell types are suitable for cardiovascular cellular therapy. Questions relating to optimal cell type, dose, mode of administration, mechanism of action, safety, and efficacy remain to be further clarified.

CHARACTERISTICS OF MESENCHYMAL STEM CELLS

MSCs were first identified in bone marrow culture (37). MSCs are stromal cells that can be isolated from most species and tissues, and in culture, MSCs are morphologically heterogeneous, containing cells ranging from narrow spindle shaped to large polygonal and slightly cuboidal cells (35). The physical property of MSCs is plastic adherence and their presence in low numbers in the bone marrow (1 out of 10^4–10^5 mononuclear cells). The capability of MSCs to differentiate into mesoderm-derived tissue and to regulate hematopoiesis has been reported (3,36). Human MSCs do not express markers of hematopoietic lineages, such as CD34, CD45, glycophorin A, T-cell, B-cell, human leukocyte antigen (HLA)-DR, CD11a, and CD14, or markers of endothelial lineage, including CD11b and CD31. MSCs do often express CD44, CD49e, CD62, CD73, CD90, CD105, CD117, CD140b, CD271, and STRO-1 (12). It is broadly accepted that the capacity for induced in vitro differentiation of MSCs into bone, fat, and cartilage is a major critical requirement in the identification of putative MSC populations (95). There is an increasing amount of data to suggest that autologous or allogeneic MSCs possess limited immune costimulatory markers and broad immunemodulatory properties that make MSCs influential on the activities of all cells involved in the immune responses (6,14,23). In addition, MSCs may act as precursor cells for stromal tissues supporting hematopoiesis and provide an enabling environment for HSC-mediated hematopoiesis, thus having a crucial role in the development and differentiation of various hematopoietic lineages through cell-to-cell interactions and by producing a number of growth factors and regulatory cytokines (36,118,119). This makes them potentially useful for various transplantation and immune-related disease treatment purposes (20). Recently, several experiments have reported that MSCs expressed several innate immune-recognized receptors, which make them sensitive to inflammatory signals, such as microbial compounds, and may be involved in inflammatory controls (94,97).

MESENCHYMAL STEM CELLS IN CARDIAC REPAIR

In Vitro Experiments

Over several years, MSCs have been intensively studied in basic cardiovascular research. Since Wakitani et al. reported that MSCs can differentiate in vitro into a myogenic phenotype, there has been a growing body of evidence that MSCs are effective in improving the cardiac performance of the IR heart (120). In vitro differentiation of MSCs into cells resembling cardiomyocytes prompted early expectation of their capacity to regenerate these cells in vivo. Exposure of the cells to a chemical 5-azacytidine (5-Aza), a DNA-methylating agent, has been the most common strategy for inducing their cardiac differentiation in vitro (5,120). The first study that mouse bone marrow-derived MSCs can be differentiated in vitro to cardiomyocyte-like cells under specific culture conditions was reported in 1999 (68). Under this condition, stromal cell lines, primary stromal cells and MSCs, from different species and different tissue sources exhibited a modified phenotype with the adoption of myotube morphology, expression of immature action potentials, and a variety of cardiac-specific genes (e.g., MEF-2A/MEF-2D) and peptides (e.g., myosin, desmin, actinin, atrial natriuretic peptides) (22,78,99,121,128). Further, functional differentiation has been indicated by the formation of gap junctions and spontaneous cell contractibility (49). Although it is not universally successful, these changes occur within 2–4 weeks of exposure to 5-Aza. Figure 1A shows that murine placenta-derived mesenchymal stem cells (PDSCs) normally exhibit a fibroblast-like morphology. By 5 days after 5-Aza treatment, the cells start to align directionally and lengthen (Fig. 1B). Approximately 40–50% of the PDSCs gradually increased in size, forming a plump appearance, or lengthened in one direction at day 10 (Fig. 1C). Finally, a myotube-like morphology in culture was formed by day 12 (Fig. 1D). Recently, in vitro alternative methods to cardiomyocyte transdifferentiation included culturing in medium enriched with dexamethasone and ascorbic acid, bone morphogenetic protein-2, and fibroblast growth factor-4, and coculture with cardiomyocytes have also been tried (98,105,132). However, it is currently unknown whether in vitro differentiation of MSCs into cardiomyocytes will enhance the reparative effects of these cells once they are transplanted in vivo.

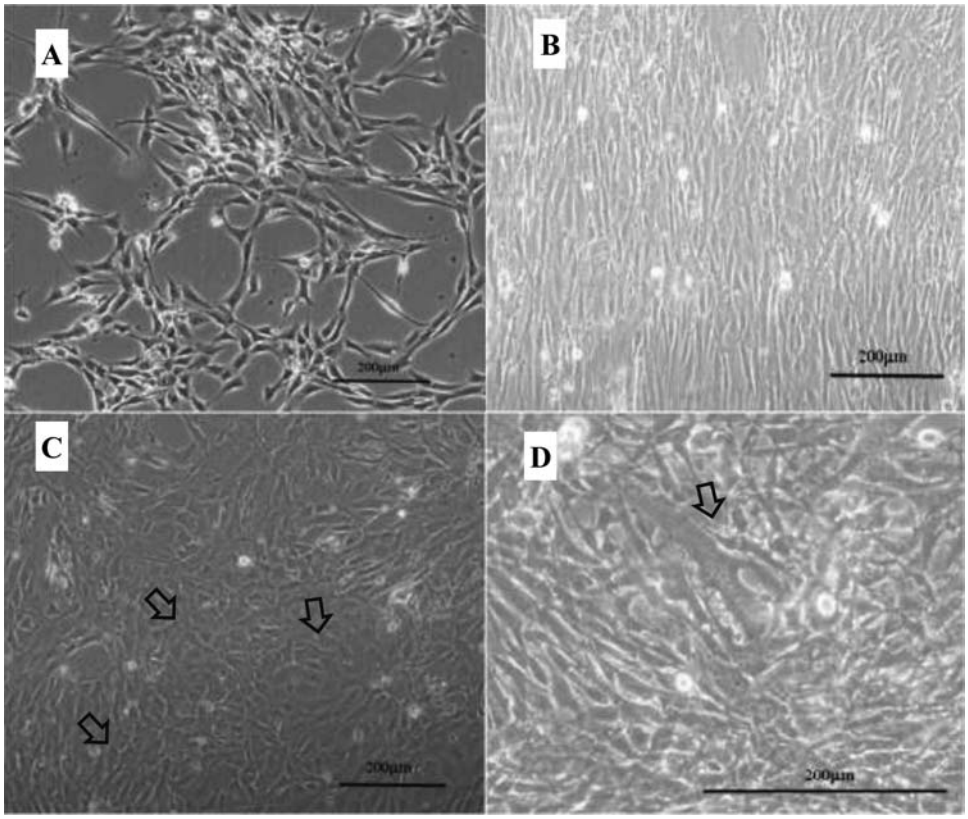

Figure 1. Phase-contrast photographs of cardiomyocyte differentiation of murine placenta-derived stem cells (PDSCs). (A) PDSCs show a fibroblast-like morphology in culture (20× objective) before 5-azacytidine (5-Aza) treatment (day 0). (B) Cells start directional alignment and lengthening at day 5. (C) Approximately 40–50% of the PDSCs gradually increased in size, forming a plump appearance, or lengthened in one direction at day 10. (D) A myotube-like morphology in culture is formed at day 12 (40× objective). Arrowhead indicates plump and myotube-like appearance.

In Vivo Experiments

Although the application of MSCs in clinical trials has been slow compared with some other cell sources, the usage of MSCs in preclinical studies has been intensively investigated. MSCs exhibit several attractive characteristics, including easy expansion in culture for transplantation, apparent potentials for mediating both myocardial and vascular repair, and hypoimmunogeneic properties enabling their usage for allogeneic transplantation. These advantages may promote their use in future clinical trials. However, knowledge on fate, efficacy, regenerative mechanisms, and safety of transplanted MSCs needs to be identified before testing them on human patients. Both large and small animal models provide proof of their functional effectiveness, pathomechanisms, and the safety of MSC transplantation (Table 2).

In 1999, Tomita et al. (114) was the first group to transplant autologous bone marrow cells (BMCs) into a rat heart at 3 weeks after cryoinjury. In this article, they identified the differentiation of transplanted BMCs in all animals by the expression of muscle-specific proteins. Moreover, functional improvement was observed only in cells that had been treated with 5-Aza. Later, in 2001, Orlic et al. (87,88) reported that direct injection of bone marrow-derived $kit^{+}Lin^{-}$ HSCs resulted in extensive myocardial regeneration in a MI mouse model. A consistent result was also reported by the Yoshioka group (131), who transplanted bone marrow-derived CD34-positive HSCs and found that they repaired infarcted myocardium in a nonhuman primate model. The first in vivo evidence for the differentiation of human MSCs into cardiomyocytes in a healthy heart was reported by the Toma group in 2002 (113). They had directly proven that purified human MSCs from adult bone marrow engrafted into the myocardium and appeared to differentiate into cardiomyocytes. Subsequently, the functional effectiveness of MSCs on MI was proven in numerous preclinical animal studies, resulting in myocardium remodeling, reduced infarct size, reduced fibrosis, and improved cardiac contractile function (2,3,32,81,104) (Table 2). These improvements were preceded by an early enhancement of resting myocardial blood flow after 1 week, which

Table 2. MSC-Based Cell Transplantation Studies in Animal Models of Myocardial Infarction

Cell Type*	Recipient Species	Time After Infarction	Outcome	Time for Follow-up	Ref.
BMCs	Rat	3 weeks	↑ Systolic and developing pressure	5 weeks	114
BMCs	Mouse	3–5 h	↓ LVEDP, ↑ LVSP, ↑dp/dt	1–2 weeks	87,88
BMCs ($CD34^+$)	Macaque	Directly	↑ FS	2 weeks	132
MSCs	Rat	7 days	↑ FS, ↓ EDV	4 weeks	3
MSCs	Pig	Directly	↑ EF, ↓ ESV	4 weeks	32
MSCs	Dog	7 days	↑ LVEF, ↑ vascularity	3 weeks	93
MSCs	Dog	13 days	↑ LVEF, ↑ vascularity, ↓ fibrosis	8 weeks	93
MAPCs	Mouse	Directly	↑ EF, ↑ FS	8 weeks	92
MAPCs	Pig	Directly	↑EF, ↑ bioenergetics	4 weeks	134
STRO-3^+ MPCs	Sheep	1 hr	↑ LVEDV, ↑ LVESV, ↑ EF	8 weeks	40

BMCs, bone marrow cells; MSCs, mesenchymal stem cells; MAPCs, multipotent adult progenitor cells; MPCs, mesenchymal precursor; dp/dt, change in systolic pressure over time; EDV, end diastolic volume; EF, ejection fraction; LVEF, left ventricular ejection fraction; ESV, end systolic volume; FS, fractional shortening (shortening of the ventricular diameter during systole; it indicates the degree of contraction); LVEDP, left ventricular end diastolic pressure; LVSP, left ventricular systolic pressure. *Unless otherwise specified, transplanted cells are from the same species. If the purity is not listed, then it is almost pure.

was confirmed by an increase in vessel size in the MSC-treated groups in comparison to control groups. These observations suggest that the transplantation of MSCs can ameliorate cardiac function by reducing infarct size, triggering neovascularization and cardiomyogenesis.

Although MSCs of various tissue origins exhibit potential effects on cellular therapy or regeneration medicine in cardiovascular diseases, it is still unknown whether a specific population of the MSCs is actually responsible for these effects. It is well known that unfractionated bone marrow stromal cells (BMSCs) or mononuclear cells (BMMNCs) contain heterogeneous adult stem cells, or progenitors, which can give rise to various mesenchymal and nonmesenchymal cell types. Nonadherent bone marrow cells expressing a chemokine C-X-C motif receptor 4^+/stem cell $antigen^+/Lin^-/CD45^-$ ($CXCR4^+/Sca\text{-}1^+/lin^-/CD45^-$) mononuclear cells in mice and $CXCR4^+/CD34^+$/antibody clone 133 (or $CD133)^+[AC133^+]/CD45^-$ mononuclear cells in humans have been reported to be capable of mobilizing into the peripheral blood and homing via chemoattraction to the infarcted myocardium (61). Most of the evidence indicated that BM-derived MSCs are $CD45^-/CD34^-/lin^-$ with $CD105^+$, $CD73^+$ (human), or $Scal\text{-}1^+$ (mice), and they contaminate populations of bone marrow cells (25). To this point, $CD45^+$-adherent stromal cells are not therapeutically effective with respect to cardioprotection in most people's views. However, we recently reported that adult mouse BMSC-derived $CD45^+/CD34^-/lin^-$ nonhematopoietic mononuclear cells were also capable of migration and engraftment into an area of infarction to achieve cardioprotection in rats by xenotransplantation (21). Mechanistically, these results indicated that CXCR4/stromal-derived factor-1 (SDF-1) and transforming growth factor (TGF)-β signals potentially enhanced the interaction of BMSCs with the damaged myocardium and increased interferon (IFN)-γ in postischemic hearts, which may cause BMSCs to behave more like stem cells in cardioprotection (21,22). Evidently, the accurate production of soluble factors TGF-β and IFN-γ in parallel with the increased expression of both TGF-β and Sca-1 receptors may favor BMSCs to achieve a more efficient protective capacity (21).

Human Clinical Trials

IR usually induces the loss of viable myocardium with the initiation of a process of adverse ventricular remodeling and a downward spiral leading to congestive heart failure. Scar tissue in the heart contributes to the incapability of the cardiac muscle to perform vital functions resulting in decreased cardiac output. Modern reperfusion strategies and advances in pharmacological management resolve the ischemia but not the infarct zone. This increases the risk of developing LV remodeling and heart failure in acute myocardial infarction (AMI) survivors. This is followed by repeated hospitalization and increased economic burden on society with 50% of the patients dying within 5 years of the diagnosis (28). Revival of the cardiac tissue in the infarct zone can enhance the functional activity of the heart. MSC-based cellular transplantation has high potential in attenuating scar formation and cardiomyocyte damage and restoring the vasculature in the infarcted area. Therefore, clinical experience and safety of MSCs aroused considerable interest (103).

Given the promising results in various animal experiments, this brings hope for using MSCs as a tool in the hands of mankind for regenerating the myocardium (Table 2). A pioneering study by Strauer et al. in 2001 showed that intracoronary transplantation of autologous stem cells 6 days after an MI was associated with a marked decrease in the infarct area and an increase in LV function

after 3 and 6 months of follow-up (107). In the following year, the same group also demonstrated that MSC injection not only improved LV function in human trials but also provided evidence on the safety and efficacy of infusing the bone marrow mononuclear fraction through the intracoronary route, albeit in a very small study population (108). Since then, there have been many published studies with different types of cells including composites of BMMNCs, EPCs, MSCs, adipose cells, and cord blood cells. To date, unfractionated adult BMMNCs are the major cells used in the clinical trials due to the following several criteria: [1] BMMNCs are easily obtained by bone marrow aspiration from large bones such as the iliac crest, [2] both precursors of cardiomyocytes and endothelial cells exist within the mononuclear cell fraction of bone marrow, [3] BMMNCs can be injected into the heart without further ex vivo expansion, and [4] the number of BMMNCs can fulfill the requirement of myocardial transplantation on great variability in the number of cells and the various routes of administration. However, there are contradictory reports as well (47). In comparison with the BMMNCs, the clinical involvement of MSCs for cardiac regeneration is in the early stages, and only a few phase I/II clinical studies have been reported (28).

Table 3 summarizes some of the BMMNC and MSC clinical trials in ischemia heart diseases. In vivo evidence of MSCs on giving rise to cardiac myocytes and vascular endothelial cells was first demonstrated in patients with acute MI following intracoronary transplantation of autologous bone marrow-derived MSCs (19). Results had shown that myocardial perfusion, LV ejection fraction, and LV chamber dimensions were significantly enhanced in a 3-month follow-up of MSC-treated patients in comparison with placebo control. Notably, this study demonstrated that MSC therapy is safe, and no deaths or arrhythmias were observed during the follow-up period. Similarly, in 2005, Katritsis et al. investigated the effects of a combination of intracoronary transplantation of BM-derived MSCs and EPCs on tissue repair in myocardial scars of patients with an anteroseptal MI (54). At a 4-month follow-up, they reported a significant decrease in wall motion score index and significant increases in myocardial viability and contractility in stem cell-treated patients compared to untreated controls. They concluded that intracoronary transplantation of MSCs and EPCs is feasible, safe, and may participate in regional myocardial regeneration following MI. In addition, the safety and feasibility of MSC therapy in old MI patients has been well described by the Mohyeddin–Bonab group (76). Eight elderly MI patients revealed smaller perfusion defect, better LV ejection fraction, and heart function enhancement without any adverse side effects.

Table 3. MSC-Based Cell Transplantation in Human Clinical Trials of Ischemia Heart Disease

Cell Type*	Numbers	Route	Delivery Day After MI	Outcome	Time for Follow-up	Ref.
BM-MNCs	$9–28\times10^{6}$	IC	7	Improved contractility and reduced infarct size	6 months	108
BM-MNCs	2.4×10^{8}	IC	3–7	Improved LVEF and reduced infarct size	12 months	4
BM-MNCs	24×10^{9}	IC	6	Improved EF and increased regional contractility	18 months	75,126
BM-MNCs	2.4×10^{8}	IC	4	Improved EF and reduced infarct size	4 months	101
BM-MNCs	$11–90\times10^{6}$	IC	10–15	Significant functional improvement and reduced infarct size	21 months	31
BM-MNCs	3×10^{8}	IC	1	Decrease scar size but no improvement in LVEF	4 months	47
BM-MNCs	8.7×10^{7}	IC	5–8	No difference	6 months	65
BM-MNCs	13.4×10^{7}	IC	6–8	Improved LV function	24 months	102
BM-MSCs	$48–68\times10^{10}$	IC	18	Increased LVEF, regional contractility, and viability of infarct zone	6 months	19
BM-MSCs	$2–4\times10^{6}$	IC	Directly	Reduced wall motion score index and improved myocardial viability and contractility	4 months	54
BM-MSCs	$2.1–9.1\times10^{6}$	IC/DI	Directly	↓ Perfusion defect and ↑ LVEF	6–18 months	76
BM-MSCs	0.5, 1.6, 5×10^{6}/kg	IV	1, 2, 3, 6 months	Improved LVEF and reverse modeling	6 months	41
BM-MSCs	$5–16\times10^{7}$	TESI	Directly	↓ Cardiac remodeling, ↓ ESV and EDV, and ↑ regional contractility	3–12 months	124
CP MSCs	$6–12\times10^{8}$	EMG	Directly	↑ LVEF and ↓ ESV and EDV	6 months	8

IC, intracoronary; IV, intravenous; BM-MNCs, unfractionated bone marrow mononuclear cells; MSCs, mesenchymal stem cells; CP MSCs, cardiopoietic mesenchymal stem cells; LVEF, left ventricular ejection fraction; EF, ejection fraction; TESI, transendocardial stem cell injection; EMG, electromechanical guidance; DI, direct intramyocardial injection.

In 2008, Osiris Therapeutics in the US announced the preliminary results of the first clinical trial using allogenic MSC transplantation for cardiac regeneration. All the MSC-treated patients exhibited improvement in the heart and lung function along with decreased arrhythmic events compared to placebo group at 6-month follow-up (79). Later, Hare et al. performed a double-blind, placebo-controlled, dose-ranging (0.5, 1.6, and 5×10^6 cells/kg) safety trial of IV allogenic MSCs (prochymal) in acute MI patients. Results of this study demonstrated a decrease in ventricular arrhythmias, enhanced pulmonary function, and increased LV ejection fraction in MSC-treated patients after 3 months (41). MSC-based cell transplantation also has remarkable therapeutic effects on patients with chronic ischemic cardiomyopathy secondary to MI. Williams et al. has recently reported that MSC-treated patients exhibited decreased cardiac remodeling and enhanced regional contractility along with decreased end-diastolic and end-systolic volumes 3 months following stem cell injection, which continued up to 1 year (124).

Most recently, the Cardiopoietic stem Cell therapy in heart failURE (C-CURE) clinical trial has reported the exciting results for the treatment of ischemic cardiomyopathy (8). In this study, MSCs were delivered to viable but defective myocardium by electromechanical guidance. At 6-month follow-up, the results showed significant enhancements in ejection fraction, compatible with improvement in end-diastolic and end-systolic volumes in the cardiopoietic-MSC therapy group in comparison to controls. Importantly, cardiopoietic-MSC therapy did not induce arrhythmias and showed no toxicity. Most groups have reported nearly identical results showing an improvement in global left ventricular ejection fraction (LVEF) and reduced LV end-systolic volume (LVESV) at 4–6 months after cell transplantation (8). Overall, the published human clinical studies demonstrated that intracoronary infusion of autologous BMSCs is safe and feasible in patients with MI. To date, a number of other clinical trial efforts in diverse places in the world are still ongoing. More up-to-date information on the progress of MSC therapy for cardiac regeneration can be obtained from http://www.clinicaltrials.gov, a web-based service by the National Institutes of Health of the US. These studies are using different interventions for applying autologous and/or allogenic MSCs in the treatment of different cardiac pathologies, such as acute MI, chronic ischemic LV dysfunction secondary to MI, and nonischemic dilated cardiomyopathy.

THE MECHANISMS OF MSC-BASED CELL THERAPY ON CARDIOVASCULAR REPAIR

Ischemic heart failure leads to cardiomyocyte death and fibrosis replacement (30). The ultimate objective for myocardial cellular transplantation is for donor cells to engraft in the recipient tissue and ultimately differentiate into new, functional cardiomyocytes and vascular cells (smooth muscle and endothelial cells). According to experimental findings and human clinical trials, the current data show that MSCs represent a suitable cell type for repair and regeneration of infarcted myocardium. MSCs have shown a number of attractive characteristics, including [1] reproducible features following isolation from different bone marrow donors, [2] high expansion potential in culture providing large numbers of cells required for transplantation within a short period of time, [3] genetic stability, [4] compatibility with tissue engineering principles, [5] possession of apparent regeneration potential in various fundamental tissues including myocardial and vascular repair, [6] homing ability to the damage tissues or inflammatory sites, [7] immune-regulatory properties that may allow their usage as an allogenic treatment, and [8] possible local (cardiac catheterization or intramuscular administration) or systemic delivery (intravenous administration).

Despite the exciting possibility that MSC therapy has major beneficial effects on myocyte repair and regeneration, including attenuation of myocardial scar and infarct size, improved regional and global ventricular function, restoration of myocardial mechanoenergetics, and increased vascular density and myocardial perfusion, contradictory results still exist, and adverse effects at myocardial sites of MSC injection have been reported in MSC transplantation. In animal studies, Yoon et al. reported that intramyocardial calcification was observed 2 weeks after injection of unselected BM cells (28.5% of rats) in rats with acute MI, but not after treatment with selected multipotent progenitor cells (130). In addition, Breitbach et al. recently reported that MSC transplantation in a murine model of cryoinfarction resulted in a high frequency of encapsulated areas containing ossifications and/or calcifications (approximately 50% of the injection sites) (16). In a human trial, Janssens et al. reported that intracoronary transfer of BMMNCs did not improve LVEF parameters (47).

There is still uncertainty about the real efficacy of MSCs on promoting the engraftment of donor cells and accelerating the speed of functional recovery of the heart. These findings highlight the need to understand the MSC tissue repair mechanisms and the exact biology of stem cells in order to address the limitations of MSC-based therapy. Several possible reasons contribute to the development of cellular cardiomyoplasty failure, including poor donor cell engraftment, short suspension, failure to differentiate, inflammatory attack-induced cardiomyocyte death, replacement fibrosis, and damage to the recipient's myocardiac microenvironment by MI. However, the molecular mechanism of this interaction in vivo is hardly certified. Four potential mechanisms might be involved in promoting myocardiac repair and functional recovery: [1] cardiac regeneration: MSCs may differentiate into cells

resembling cardiomyocytes; [2] cardiac repair: paracrine effect; [3] niche contribution: MSCs are components of the myocardium cell niche; therefore, MSCs provide for not only the renewal of stroma, but they also help maintain the niche for cardiac stem cell/progenitor homeostasis; and [4] cardiac remodel regulation: the immunomodulatory effects of MSCs have offered greater possibilities of allogeneic sources of MSCs in the management of immune rejection and inflammatory regulation of MSCs on cardiac repair and regeneration. Most likely, all of these mechanisms work together in an orchestra of factors to regulate stem cell function (Fig. 2).

Cardiac Regeneration by Cardiomyocyte Differentiation

The mammalian heart possesses little intrinsic capacity for regeneration; therefore, recovery of the cardiac tissue in the infarct region can enhance the functional activity of the heart. The evidence for MSC's capability to differentiate into cardiomyocytes can be proven by the expression of the myotube phenotype, cardiac-specific

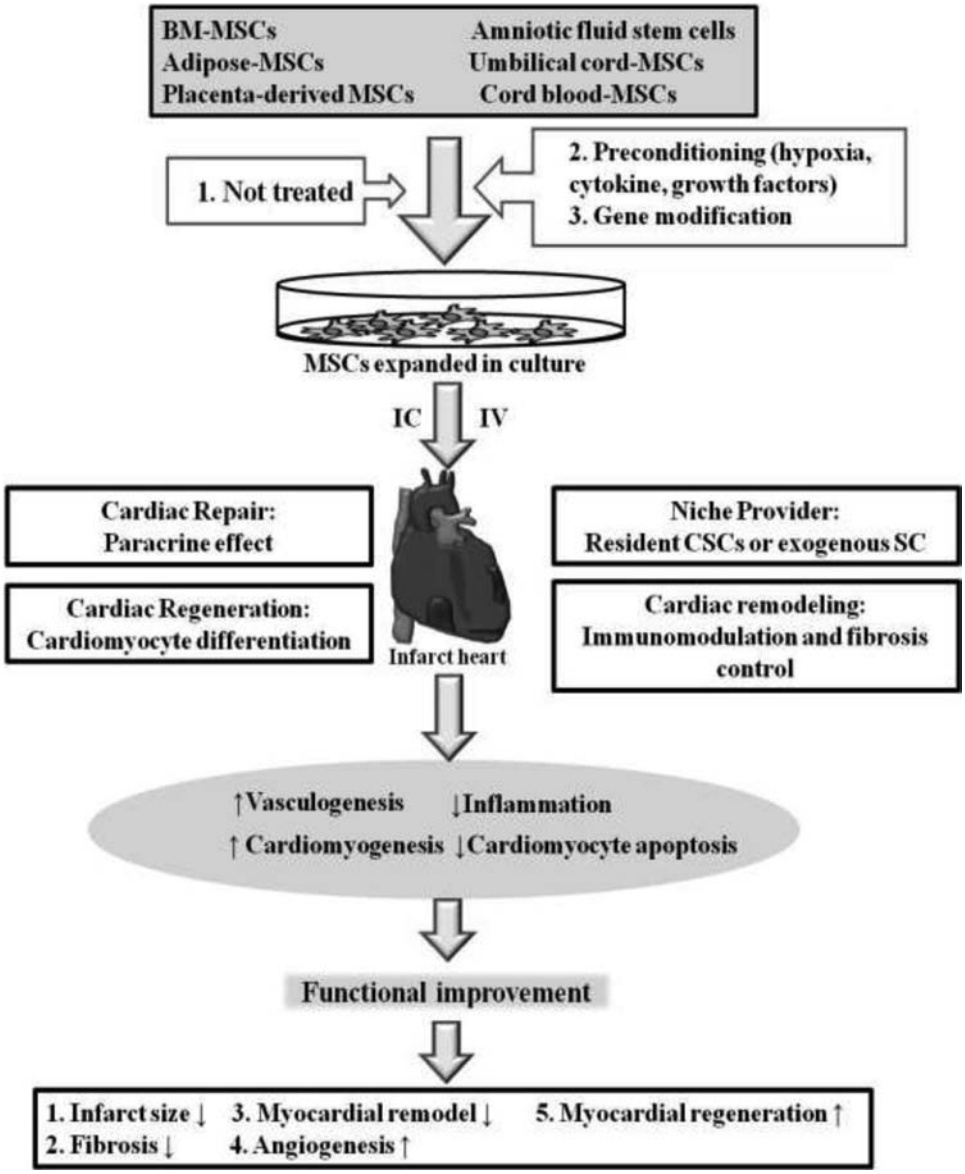

Figure 2. The mechanisms of MSCs for cardiac functional improvement. Through cardiac regeneration, paracrine mechanism, niche providing, and inflammatory control, MSCs can reduce infarct size, fibrosis, and myocardial remodeling, then increase angiogenesis and myocardial regeneration. Abbreviations: MSC, mesenchymal stem cells; CSC, cardiac stem cells; SC, stem cells; IV, intravenous; IC, intracoronary.

genes and proteins, intercellular connections via intercalated disc, and spontaneous cell contractility (21,49, 68,78,99,128). Moreover, MSC-differentiated cardiomyocytes can produce cardiac connexin-43, which can electromechanically couple to host cardiomyocyte in vivo (115). Evidence for in vivo differentiation has come from immunohistological analysis in small animal studies of xenogeneic MSC transplantation (70,113) and large animal studies of autologous or allogeneic cell therapy (2,104,106). Generally, authentication of differentiation on those studies has been incomplete. Several groups have reported that engrafted BM-derived cells actually undergo fusion with endogenous cardiac cells, rather than true differentiation (1,82). To date, the clear evidence for in vivo differentiation observed with MSC therapy requires concern for the following: [1] whether to use the impure heterogeneous cell population obtained through plastic adherence isolation; [2] whether MSC expansion ex vivo in media should contain xenogeneic ingredients such as fetal bovine serum; and [3] if biological activities of MSCs are in extremely modest rates of intramyocardial retention, engraftment, and survival of cells following their administration by current delivery techniques. Cell survival may be especially compromised in the presence of unresolved myocardial ischemia or inflammation. However, there is still controversy as to whether actual differentiation occurs versus large cell fusion with resident myocytes. This is because of the myocyte deficiency in infarction-induced heart failure.

Cardiac Repair Through Paracrine Effects

It is evident that following transplantation of hundreds of millions of cells, less than 2% of the cells are actually still present in the tissue within 2 weeks of implant. On the other hand, the functional LVEF improvement that occurred within 72 h was far earlier than would be expected for cell regeneration, leading to intense debate about repair mechanisms after cell transplantation (79). It is believed that current cell therapies assist the heart predominantly by facilitating endogenous repair processes, rather than through actual regeneration of lost cardiac and vascular cells. The prevailing concept of stem cell efficacy has now shifted toward paracrine effects, by which stem cells have been shown to modulate angiogenesis, cytoprotection from apoptotic cell death, induce proliferation of endogenous cardiomyocytes, and may recruit resident cardiac stem cells (39).

MSCs secrete a variety of cytokines and growth factors that have both paracrine and autocrine activities (17). Experimental results have suggested that hematopoietic cytokines are actively involved in the pathophysiology of several cardiovascular disorders, including ischemia/reperfusion injury, vascular remodeling, and chronic heart failure (90). Moreover, both in vitro and in vivo studies showed that MSCs/progenitor cells secrete growth factors including SDF-1/chemokine C-X-C motif ligand 12 (CXCL12), hepatocyte growth factor (HGF), insulin-like growth factor-1 (IGF-1), basic fibroblast growth factor, hypoxia inducible factor-1 α (HIF-1α), vascular endothelial growth factor (VEGF), angiopoeitin-1, monocyte chemoattractant protein-1 (MCP-1), interleukins-1 and -6, placental growth factor, plasminogen activator, and tumor necrosis factor-α (52,57,127). In fact, ex vivo pretreatment of autologous bone marrow-derived progenitor cells with cardiomyogenic growth factors boosts their cardiac differentiation and their functional regenerative capacity in vivo (7). Alternatively, our previous study has shown that the accurate production of soluble factors SDF-1, TGF-β, and INF-γ in parallel with increased expression of their receptors may favor BMSCs to achieve a more efficient cardioprotective capacity (21). Paracrine actions of MSCs may underlie much of this reparative potential, including the capacity for cell transplantation to induce neovascularization, reduction in infarct size and scar formation, and improvement in myocardial contractility (57). Secreted molecules might act via direct effects on the MSCs themselves or indirectly by inducing other cells nearby, followed by alteration in their biological properties and functions.

Niche Provider: MSCs Are Components of Myocardium Cell Niche

MSCs provide not only the renewal of stroma, but they are also a niche dweller of HSC homeostasis. However, it remains unclear whether MSCs from other tissues are part of the niche for tissue-specific resident stem cells or cells near to them. It is also not clear what the targets of MSC-mediated paracrine effects are, but probably include both mature cells and resident progenitor cells in the recipient heart. It has been reported that MSCs isolated from myocardium were found to be capable of stimulating the survival and proliferation of cardiac stem cells in vitro (66); however, the questions of whether and how these cells interact with tissue-specific stem cells in vivo remains unclear (71).

The presence of resident cardiac stem cells was first reported by Beltrami et al. in 2003 (10). Cardiac stem cells (CSCs) appear to reside in specialized niches, which support the growth and maintenance of the stem cell pool (77). Experimental studies have reported that the postnatal heart possesses a niche of CSCs or cardiac progenitors with the capacity to replicate and differentiate into cardiac myocytes (9,58). More evidence has shown that there is a marked increase in the number and migration of such cells to injury areas following an ischemic insult (83). In addition, CSCs isolated from the heart ventricles of rat subjects have been shown to be effective in the treatment of myocardial ischemia, therefore making the heart a viable source of stem cells for myocardial repair (24).

Although the different cardiac stem cell pools are small relative to the mature resident cardiomyocytes, they are believed to be the source of new cells in normal organ homeostasis as well as in stressed myocardium. This new finding not only challenged our understanding of the self-renewing characteristic of the heart but also opened a new therapeutic option for cardiovascular diseases.

To date, little is known about the anatomical location, architecture, and cellular composition of the myocardium niche. It is well accepted that self-renewal and differentiation of tissue-specific stem cells have to be tightly regulated by the appropriate stem cell niche via cell-to-cell contact or paracrine growth factors secreted by niche cells. A concept has been proposed, that there are two types of MSCs in the body: exogenous MSCs circulating in the blood, which participate in tissue repair upon injury, and resident MSCs, which are located in the perivascular region of all organs and tissues of the body and regulate physiological tissue renewal and the maintenance of tissue homeostasis (51).

Cardiac Remodel Regulation: Inflammatory Control

Pathological remodeling is associated with the replacement of native cells by fibrotic tissue that reflects poor clinical prognosis and a stage of irreversibility. The pathological tissue-remodeling process is characterized by an accumulation of mainly type I collagen and collagen-producing fibroblasts, and this progresses until it finally results in cardiac fibrosis (69). Enhanced fibrosis leads to stiffening of the ventricles and impaired diastolic filling that indicates the irreversible end stage of the heart disease. Myocardial inflammation promotes ongoing myocardial fibrosis, which is a hallmark of end-stage heart failure. The hostile microenvironment affects the function of tissue-specific stem and progenitor cells and possibly also resident CSCs. It is expected that excessive inflammation promotes angiogenesis and affects survival of resident cells, such as endothelial cells, cardiomyocytes, as well as inflammatory cells in the myocardium. Hence, current evidence supports the view that the unhealthy microenvironment, such as inflamed myocardium, prevents the cardioregenerative potential of stem cells in the infarcted heart. Instead, this nonphysiological microenvironment promotes generation of monocytes and myofibroblasts and their participation in scar formation after myocardial injury (43,53,116).

It has been demonstrated that administration of $CD133^+$ progenitors into the myocardium at different stages of experimental autoimmune myocarditis (EAM) resulted in different cellular phenotypes (53). Diverse stimulants, such as cytokines, chemokines, apoptotic and necrotic cells, and extracellular matrixes are discretely existent in the healthy, inflamed, and fibrotic myocardium and create a unique myocardial signaling milieu. Apparently, the microenvironment of the inflamed and fibrotic heart promotes the fibroblast fate of inflammatory progenitors. There is an emerging amount of data to suggest that autologous or allogeneic MSCs possess broad immune-modulatory properties that make MSCs influence the activities of all cells involved in the immune responses. In more than 95% of MSCs, the immune phenotypes are expressed at low levels of major histocompatibility complex (MHC) class I, but no MHC class II. MSCs lack surface expression of Fas-ligand or costimulatory molecules, such as B7-1, B7-2, CD40, or CD40L. In addition, they do express adhesion molecules involved in T-cell interaction, including vascular cell adhesion molecule (VCAM-1), intercellular adhesion molecule (ICAM), and lymphocyte function-associated antigen 3 (LFA-3; CD58) (67). In the absence of costimulation, T-cell and MSC engagement can result in anergy.

In vitro studies have shown that MSCs dose-dependently suppresses lymphocyte proliferation in mixed lymphocyte culture, induced by alloantigens, mitogens such as phytohemagglutinin (PHA), concanavalin A (Con A), tuberculin, as well as activation of T-cell by CD3 and CD28 antibodies (6,26,64). MSCs also affect monocytes on maturation or functional operation. In addition, MSCs decrease IFN-γ, interleukin (IL)-12 and tumor necrosis factor (TNF)-α production after coculture with monocytes (14). The immunomodulatory effects of MSCs have offered greater possibilities of allogeneic sources of MSCs in the management of immune rejection, and inflammatory regulation of MSCs on cardiac repair and regeneration is being tested in a prospective, randomized trial. The study of Kania et al. demonstrated that the protective function of $CD133^+$ progenitor cells depends on the nitric oxide-mediated suppression of heart-specific $CD4^+$ T-cell expansion (53). Furthermore, MSC transplantation prevents heart failure development and improves cardiac function by inducing neovascularization and by inhibiting inflammatory cytokine production (84,85). To this end, modulation of the myocardial microenvironment might prevent the inflammatory stem/progenitor cells into pathological fibroblasts. Such strategies may give rise to stem cell-targeted therapies against fibrotic processes associated with pathological cardiac remodeling.

CONCLUSIONS

Experimental findings and clinical trials have proven the feasibility, safety, and efficiency of MSC therapy for cardiac regeneration, but there is still uncertainty about the real efficacy of MSCs on promoting engraftment of donor cells and accelerating the speed of cardiac recovery. Despite the exciting possibilities that stem cell therapy has major beneficial effects on myocyte regeneration, inconsistent outcomes and, in some cases, poor engraftment, donor cell apoptosis, and modest improvement have been reported in human trials.

The promising therapeutic effect(s) of MSCs is dependent on their capacity to engraft and survive in the target tissue. However, transplantation of large amounts of cells into infarcted hearts yields only marginal improvement in cardiac functions. This is possibly due in part to poor donor cell engraftment, low retention of stem cells, or increased immunogenicity of the transplanted cells in an ischemic environment. Therefore, strategies to enhance repair cells implanted into the heart by preconditioning stem/progenitor cells to certain physical or chemical culture conditions, genetically modified stem cells, or to decrease immune rejection of transplanted cells by the recipients' immune system are key to the successful treatment of cellular therapy. In addition, the focus of improving and standardizing cell processing and delivery methods should be on enhancing cell engraftment while maintaining their therapeutic potential. Recently, it has been reported that cardiomyocyte survival in IR subjects is increased by previously repeated exposure to hypoxia or sublethal ischemia (13,80). In addition, preconditioning with certain tissue protective factors enhances survival and engraftment of the transplanted cells and further triggers additional host repair cells into the injured heart (91). Moreover, fetal priming with donor BMSCs may enhance the efficiency of stem cell-mediated cardiac protection after a second boost in rat IR model (21). However, detailed mechanism(s) underlying preconditioning or priming in cellular therapy remain(s) to be elucidated.

MSCs and mesenchymal lineage precursors play an important role in normal vascular development and may be derived from a subset of smooth muscle cells. These results highlight the need to understand the MSC tissue repair mechanisms and the exact biology of stem cells in order to address the limitations, such as the optimal cell type, mode of cell processing, and delivery. The mechanisms and molecules involved in MSC functions still remain unconfirmed. Current data suggest that MSCs exhibit three key features that could have a profound impact on their clinical use. First, MSCs are able to be saved or directly differentiated into cardiomyocytes to replace the damaged site of heart. Second, MSCs can enable immune cells to stay in an "anergy" state through immune-related mechanisms to prevent ongoing myocardial fibrosis. Third, MSCs could create an accessible microenvironment to repair the damaged myocardium and provide trophic factors to support the survival of tissue-specific stem/progenitor cells, and this largely recapitulates their physiological effect in the niche. To date, MSCs have been employed in the clinical setting in phase I/II clinical trials. However, longer follow-up is necessary to draw definitive conclusions on late potential adverse events.

ACKNOWLEDGMENTS: The authors acknowledge financial support from National Science Council (NSC 101-2314-B-030-004, NSC 102-2314-B-030-007) and also supported in part by Taiwan Department of Health Clinical Trial and Research Center of Excellence (DOH102-TD-B-111-004). The authors declare no conflicts of interest.

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