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Repair mechanisms of bone marrow mesenchymal stem cells in myocardial infarction

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Abstract

The prognosis of patients with myocardial infarction (MI) and resultant chronic heart failure remains extremely poor despite advances in optimal medical therapy and interventional procedures. Animal experiments and clinical trials using adult stem cell therapy following MI have shown a global improvement of myocardial function. Bone marrow-derived mesenchymal stem cells (MSCs) hold promise for cardiac repair following MI, due to their multilineage, self-renewal and proliferation potential. In addition, MSCs can be easily isolated, expanded in culture, and have immunoprivileged properties to the host tissue. Experimental studies and clinical trials have revealed that MSCs not only differentiate into cardiomyocytes and vascular cells, but also secrete amounts of growth factors and cytokines which may mediate endogenous regeneration *via* activation of resident cardiac stem cells and other stem cells, as well as induce neovascularization, anti-inflammation, anti-apoptosis, anti-remodelling and cardiac contractility in a paracrine manner. It has also been postulated that the anti-arrhythmic and cardiac nerve sprouting potential of MSCs may contribute to their beneficial effects in cardiac repair. Most molecular and cellular mechanisms involved in the MSC-based therapy after MI are still unclear at present. This article reviews the potential repair mechanisms of MSCs in the setting of MI.

Keywords: bone marrow • mesenchymal stem cells • myocardial infarction • repair mechanisms

Introduction

Cardiovascular disease (CVD), especially myocardial infarction (MI) with resultant congestive heart failure (CHF), is a leading cause of mortality and morbidity worldwide [1]. Despite recent improvements in disease prevention and combinative therapy (medical, interventional, device and transplantation) for MI and CHF, the 1 year mortality rate for patients with acute MI and impaired left ventricular function is still only 13% [2]. Furthermore, CHF is associated with a 20% mortality rate per year [3]. The emergence of stem cell therapy may represent a promising outlook for patients with CVD. Since Makino and his colleagues induced cardiomyocytes (CMCs) from bone marrow stromal cells by 5-azacytidine treatment *in vitro* in 1999 [4], several types of stem cells have been used in an explosive manner for studies on cardiac cell repair therapy in animal and clinical experiences. For example, pluripotent embryonic stem cells, bone marrow adult stem cells, peripheral tissues adult stem cells and adult stem cells from the heart itself have been used in these myocardial stem cell repair therapy studies.

Mesenchymal stem cells (MSCs) with no consensus definition are currently defined by their ability to adhere to the surface of cell culture dishes and the absence of haematopoietic markers. MSCs can be easily isolated and expanded in culture, and can be induced to differentiate into chondrocytes, adipocytes, myocytes and CMCs *in vitro* and undergo site-specific differentiation *in vivo* [5, 6].

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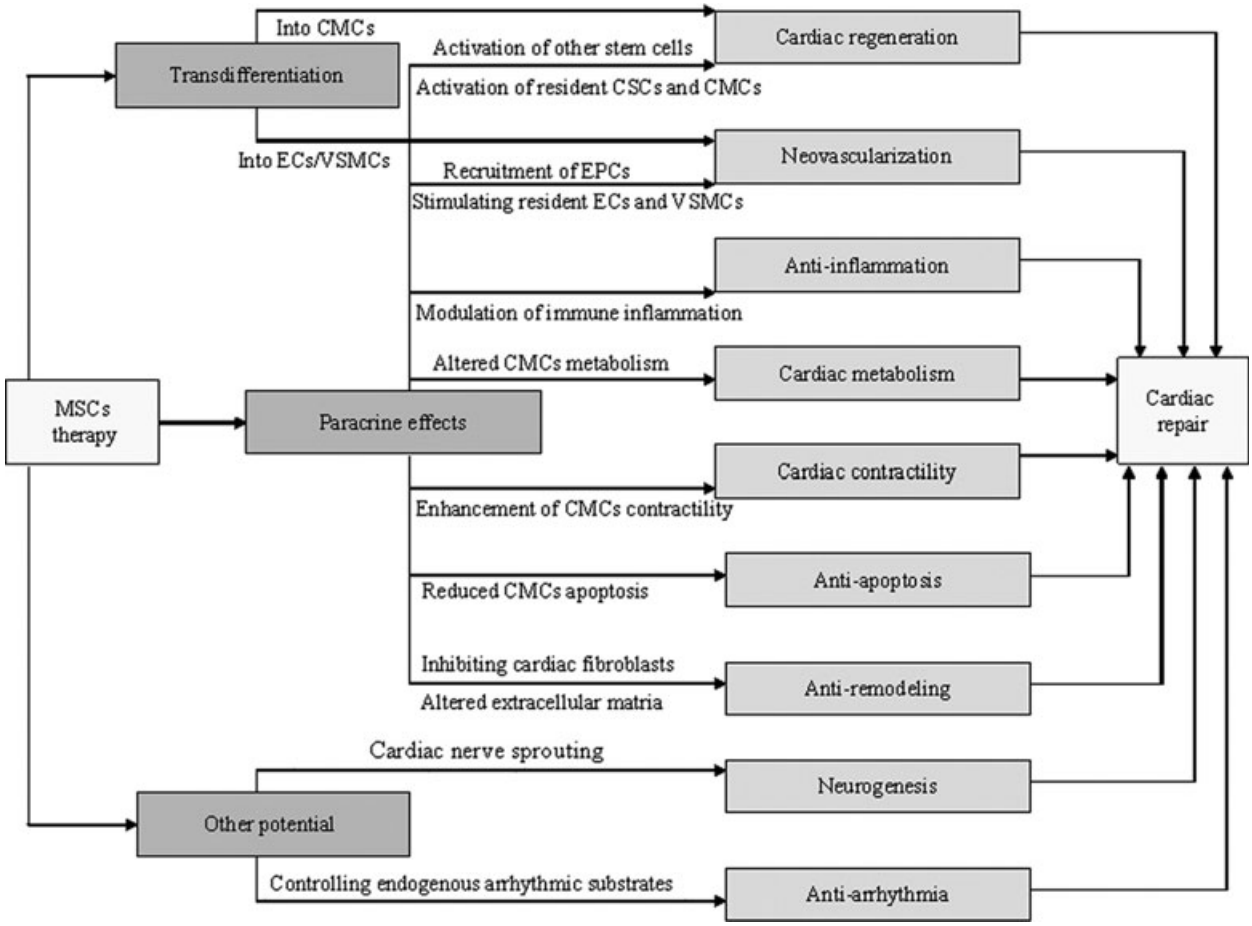

Fig. 1 Proposed repair mechanisms of bone marrow MSCs in MI. Transdifferentiation of MSCs into CMCs and vascular cells leads to cardiac regeneration and vasculogenesis. MSCs can exert actions on different cell types, leading to endogenous cardiac regeneration, neovascularization, anti-inflammation, anti-apoptosis, anti-remodelling, cardiac contractility and cardiac metabolic modulation in a paracrine manner. Neurogenesis and anti-arrhythmia may also contribute to cardiac repair in MSC-based therapy. MSCs: mesenchymal stem cells; CMCs: cardiomyocytes; CSCs: cardiac stem cells; EPCs: endothelial progenitor cells; ECs: endothelial cells; VSMCs: vascular smooth muscle cells.

In the last decade scientists have observed that MSCs maintain their multilineaged capacity after expansion and transplantation, and seem to have unique immunological characteristics that allow persistence in a xenogeneic environment. This makes them a promising source for cell therapy in the setting of MI with subsequent CHF. We have observed that administration of MSCs by intravenous, intraventricular or intramyocardial injection can improve myocardial function before and after cardiopulmonary resuscitation (CPR) and duration of survival after CPR in MI rats [7, 8].

Recent studies in clinical experiences have revealed that MSC therapy is safe and may improve cardiac function and structural remodelling in patients with acute MI or CHF [9]. However, the mechanism of beneficial effects from MSC-based therapy for MI is yet to be understood. Multiple biological mechanisms, such as cardiac regeneration, neovascularization, paracrine effect and immunoregulation, and others, may contribute to the efficacy of MSC therapy in acute MI and after MI (Fig. 1). This review focuses on experimental studies and clinical trials with MSCs derived from bone marrow unless specially declared herein, and provides an overview of current knowledge of the underlying mechanisms contributing to their efficacy in therapy for MI.

Transdifferentiation

MSCs differentiate into CMCs

MI leads to a significant loss of cells and formation of scar tissue. The remaining CMCs are unable to reconstitute the necrotic tissue, and cardiac function deteriorates during the ensuing course. Orlic

and his colleagues, in their paper published in *Nature* in 2001, indicated that locally delivered bone marrow cells could generate *de novo* myocardium *in vivo* in infarcted mice [10]. Since then, several types of stem cells, including MSCs, have been used for cellular cardiomyoplasty following MI. MSCs can be isolated from adult bone marrow and may be induced to differentiate into CMCs both *in vitro* [4, 11] and *in vivo* [5, 12]. Transplantation of MSCs by injection into the myocardium [12] or through the tail vein [13] or other administration [14], shows positive cardiac markers, such as desmin, cardiac troponin T, sarcomeric α-actinin or connexin43 in infarcted myocardium. MSCs can also achieve long-term survival, engraftation, and trilineage differentiation following transplantation into chronically scarred myocardium [14]. Furthermore, Fukuda *et al.* have even proved that it is mesenchymal, rather than haematopoietic stem cells derived from bone marrow that can differentiate into CMCs after transplantation in infarcted mice [15]. However, it has been shown that human MSCs engrafted in a xenomodel can express a CMC protein but cannot regenerate contracting CMCs due to failure of full differentiation, despite improvement in cardiac performance after transplantation [16]. This has also been confirmed in some experiments *in vitro* [17]. Furthermore, Silva *et al.* revealed that improvement of cardiac function resulted from increased vascularity by vascular smooth muscle cells (VSMCs) and endothelial cells (ECs) differentiated from MSCs, but no myocytes were detected in chronic ischemic canines [18]. Grinnemo *et al.* implanted human MSCs into a cardiac ischemic xenomodel and observed no CMC differentiation, accompanied by no improvement in myocardial function in treated animals compared to controls [19].

Factors that can formulate and influence MSCs to differentiate into CMCs are unclear at present. It is presumed that cardiac environment may influence the potential of MSCs to differentiate into CMCs, because infarcted myocardium may not only influence MSCs homing to and retention in the heart, but may also influence transplanted MSCs morphology and mobility within the heart. They even mediate their site-specific differentiation, despite that only a small number of cells are actually within the infarct zone due to the hostile environment [5, 20, 21]. MSCs co-cultured with neonatal ventricular myocytes have the potential to transdifferentiate into CMCs, which confirms that MSCs possess the potential to differentiate into functional cardiac phenotypes by cardiac microenvironment [22]. Furthermore, infarct-related biological and physical factors following MI induce commitment of human cord blood MSCs to CMC-like cells through transforming growth factor-1β (TGF-1β) and bone morphogenetic protein-2 (BMP-2) pathways, which are significantly greater in myocardium following MI than in normal myocardium [23]. Growth factors, such as vascular endothelial growth factor (VEGF), insulin-like growth factor-1(IGF-1) and myocardin can influence and enhance MSCs capability to differentiate into more CMCs, resulting in further improvement in myocardial perfusion and in the restoration of heart function [24–26]. The hypoxic microenvironment of infarcted myocardium may be a strong mediator to activate MSCs to increase the expression of these factors, contributing to more differentiated CMCs. For example, hypoxia can activate MSCs to increase VEGF, fibroblast growth factor (FGF)-2, hepatocyte growth factor (HGF) and IGF-1 expression through a nuclear factor (NF-κB) dependent mechanism [27]. Wang and his colleagues have also demonstrated that it is direct cell-to-cell contact between MSCs and adult CMCs, but not the soluble signalling molecules, that obligates the differentiation of MSCs into CMCs [11]. It has also been revealed that the differentiation of MSCs to CMCs, VSMCs, and ECs may be time dependent [13]. Further research is required to devise an optimal method for visualizing MSCs differentiating into CMCs after transplantation into the host myocardium. Cell-to-cell signals and other influential factors involved in this process should also be completely elucidated in future studies. In addition, future work should assess the potential of unwanted differentiation from MSCs and tumorigenesis promoted by MSC-derived CMCs in order to prevent deleterious effects of cell transplantation after infarction.

MSCs differentiate into vascular cells

It is reasonable to anticipate that the generation of a network of capillaries and larger size blood vessels for supply of oxygen and nutrients to both the ischemic and *de novo* myocardium is as important as the regeneration of functional CMCs in MSC-based therapy after MI. Recent studies have revealed that MSCs can differentiate into angioblasts, including ECs and VSMCs due to their multilineage differentiation potential. Some evidence has revealed that transplanted MSCs in the MI area have significantly higher expression rates of CD31, von Willebrand factor and smooth muscle (SM)-actin, accompanied by increase in capillary density, resulting in improvement in cardiac performance [13, 18]. Dai *et al.* have also revealed in their studies that allogeneic MSCs can survive in infarcted myocardium for up to 6 months and express markers that suggest muscle and endothelium phenotypes, despite the transient benefit of improvement in global ventricular function likely *via* a paracrine manner [28]. The transdifferentiation potential of MSCs into ECs and VSMCs has also been proved in many experiments *in vitro*. For example, Oswald *et al.* revealed the differentiation of expanded adult human MSCs into cells with phenotypic and functional features of ECs *in vitro* [29].

Just as their influence on CMCs transdifferentiation, some factors may also influence MSC differentiation into ECs and VSMCs. Some evidence has revealed that transplanted MSCs are preferentially attracted to the infarcted, but not the non-infarcted, myocardium [21], which implies that pathological and physiological changes in infarcted myocardium may be influential factors on transdifferentiation of ECs and VSMCs by MSCs, after transplantation. Stromal cell-derived factor-1α (SDF-1α) is an important mediator of stem cells homing to the injured heart. SDF-1 is predominantly localized in the MI lesion, matched with increased accumulation of MSCs and an improvement in cardiac function after MSC therapy, but its effect on MSC migration is almost completely blocked by phosphoinositide 3-kinase (PI3K) inhibitor and CXC chemokine receptor (CXCR)4-specific antagonist [30]. The authors of this study concluded that SDF-1 with its receptor,

CXCR4T, might mediate the migration of MSCs towards MI heart tissue through activation of PI3K/Akt. Furthermore, SDF-1α can induce MSC differentiation into ECs *in vitro*, and its overexpression can enhance this efficacy in the infarcted heart, coupled with increased expression of VEGF, Akt and endothelial nitric oxide synthase (eNOS), whereas nitric oxide synthesis inhibitor can inhibit these effects both *in vitro* and *in vivo* [31]. Hypoxic conditions can activate MSCs to increase VEGF, HGF and IGF-1 expression. Their enhanced expression may significantly enhance angiogenesis after MSC therapy, at least partly through inducing proliferation of ECs and α-smooth muscle actin$^+$ cells from transplanted MSCs [32–34]. Recent studies have revealed that direct cell-to-cell contact between MSCs and angioblasts, transplantation time and donor age, and other factors, may sometimes pay a pivotal role in the differentiation of MSCs to ECs and VSMCs [11, 13, 35, 36]. Therefore, in order to maximize the efficacy of angiogenesis by MSC transdifferentiation into angioblasts after MI, further experiments are required to achieve deeper insight into the pathways and molecules involved in this process. The association between MSC-derived vascular cells and potential risks for adverse effects, such as in-stent restenosis and atherosclerosis, is debatable, thus detailed identification of this association warrants further research.

Paracrine effects

In most cases, the incidence of myocardial and vascular regeneration, either by transdifferentiation or cell fusion, appears too minor to explain the significant recovery of cardiac function. Recent studies have demonstrated that MSCs can secrete growth factors and cytokines which can exert their influence on cardiac repair in a paracrine fashion, after MI [37, 38], despite no apparent paracrine effect on the growth behaviour of the surviving myocardium, or a local but not a functional effect observed in some studies [39, 40]. Under hypoxic conditions, MSCs can release growth factors and cytokines VEGF, FGF-2, FGF-7, HGF, IGF-I, TGF-1β, secreted frizzled related protein 2 (Sfrp2), angiopoietin-1(Ang-1), SDF-1α, matrix metalloproteinase-9 (MMP-9), interleukin-6 (IL-6), IL-1, tumour necrosis factor (TNF-α) and others [37, 38, 41, 42]. These release growth factors and cytokines can promote cardiac endogenous regeneration and neovascularization, as well as induce anti-apoptotic, anti-inflammatory and anti-remodelling effects *via* interlinked molecular signal pathways in a paracrine manner [37, 43] (Fig. 2).

Endogenous cardiac regeneration induced by paracrine effects

It has been shown that the heart contains stem cell niches which can be influenced by implanted MSCs to restoration through multifaceted cell-to-cell interactions [44]. Cardiac stem cells (CSCs), including side population cells, c-Kit$^+$ cells, Sca-1$^+$ cells, cardiospheres cells and Isl1$^+$ cells, residing in the heart, are self-renewing, clonogenic and multipotent, and may be induced to give rise to CMCs [43]. Hatzistergos and his team have recently proved that MSCs may stimulate endogenous CSCs, including c-kit$^+$ CSCs and GATA-4$^+$ CSCs, proliferation into enriched populations of adult cardioblasts that express Nkx2–5 and troponin I both *in vivo* and *in vitro* [45]. The heart has an endogenous reserve of CSCs possessing growth factor receptor systems that may be activated by growth factors, such as VEGF, HGF and IGF-1, to reconstitute dead myocardial tissue and recover cardiac function [46–48]. MSCs may be used as a trigger to induce resident CSCs, as well as other stem cells to retrieve dead myocardial tissue after infarction *via* releasing paracrine factors. For example, Zisa *et al.* have proved that the trophic factors from MSC-conditioned medium are responsible for cardiac regeneration, and VEGF may be a key therapeutic trophic factor in MSC-mediated cardiac repair independent of MSC differentiation or stemness [49]. VEGF may induce CSCs migration *via* activation of PI3K/Akt in a concentration-dependent manner, and its role of homing CSCs is inhibited by either the VEGF receptor blocker or the PI3K/Akt inhibitor [46]. It has been shown that the SDF-1 gradients from bone marrow to blood and from blood to myocardium increase after MSC transplantation in comparison with saline treatment, accompanied by an increase in cells positive for CD34, CD117 and STRO-1 in the infarcted myocardium [50]. SDF-1α and TGF-β may play pivotal roles in the mobilization and differentiation of marrow-derived progenitor cells [51] and CD117$^+$ stem cells [52] in the injured adult heart, resulting in endogenous regeneration and cardiac functional recovery. Endogenous cardiac regeneration from resident CSCs may avoid unwanted differentiation and malignant proliferation, coupled with fewer side effects. Therefore, the use of conditioned medium of MSCs rather than MSCs alone may be a viable option for efficient cardiogenesis in future research. However, more research is required to precisely ascertain the course of endogenous cardiac regeneration occurring after MSC transplantation in the host myocardium.

Neovascularization induced by paracrine effects

Neovascularization, including vasculogenesis, angiogenesis and arteriogenesis, is another important biological process positively influenced by MSCs in cardiac repair after MI. Recent evidence has demonstrated that under ischemic conditions, MSCs release pro-angiogenic and pro-arteriogenic cytokines, such as nitric oxide, VEGF, basic fibroblast growth factor (bFGF), IGF-1, Ang-1 and others. These secrete molecules, which may home and induce endothelial progenitor cells (EPCs) differentiation into ECs and VSMCs, induce ECs sprouting from pre-existing blood vessels and induce ECs and VSMCs maturation in a paracrine manner [37]. Tang *et al.* have demonstrated that paracrine action enhances the effects of autologous MSC transplantation on vascular regeneration in rat model of MI [53]. They found that angiogenic factors bFGF, VEGF and SDF-1α increased in the MSC-treated hearts compared with medium-treated hearts, accompanied by increase in capillary density. Some evidence reveals that oestrogen affects the

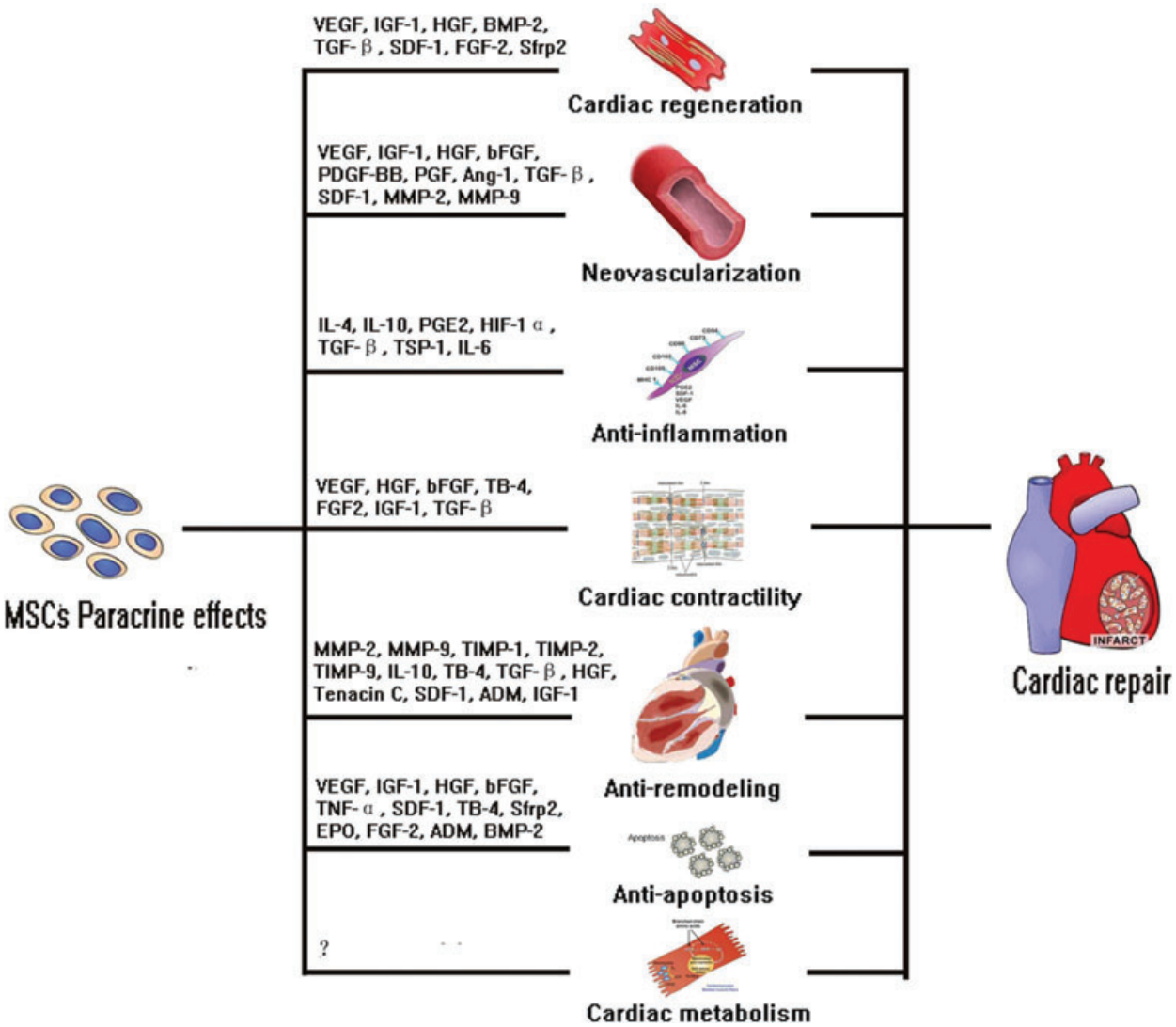

Fig. 2 Summary of MSC-secreted paracrine factors. MSCs release soluble factors that markedly alter the myocardial microenvironment in response to specific environmental stimuli after infarction. These biologically active molecules exert paracrine actions on a variety of different cell types in many processes of cardiac repair, including cardiac regeneration, neovascularization, inflammation, apoptosis, remodelling, contractility and metabolism. VEGF: vascular endothelial growth factor; FGF: fibroblast growth factor; HGF: hepatocyte growth factor; IGF: insulin growth factor; Ang: angiopoietin; PDGF: platelet-derived growth factor; SDF: stromal cell-derived factor; PGF: placental growth factor; TGF: transforming growth factor; ADM: adrenomedullin; Sfrp: secreted frizzled related protein; TB4: thymosin β_4; BMP: bone morphogenetic protein; TNF: tumour necrosis factor; IL: interleukin; MMP: matrix metalloproteinase; TIMP: tissue inhibitor of matrix metalloproteinases; bFGF: basic fibroblast growth factor; PGE: prostaglandins E; HIF: hypoxia-inducible factor; TSP: thrombospondin; EPO: erythropoietin.

recovery of ischemic myocardium partially through paracrine growth hormone produced by MSCs and facilitation on mobilization of EPCs to the ischemic myocardium, partly *via* eNOS-mediated activation of MMP-9 [54, 55]. It has been shown that MSCs can enhance EC proliferation and sprout formation [56]. Several factors, such as VEGF, TGF-1β and others, released from MSCs, can promote angiogenesis by stimulating EC sprouting. Beckermann *et al.* demonstrated that VEGF within the supernatant from subconfluent MSCs increased sprouting of ECs as detected by reverse transcriptase-polymerase chain reaction and enzyme linked immunosorbent assay [57]. However, another study observed conditioned media from mechanically stimulated MSCs in two-dimensional tube formation and three-dimensional spheroid sprouting assays revealed enrichments of MMP-2, TGF-1β and bFGF, but not of VEGF [58]. The underlying mechanism of MSCs regulating angiogenesis according to their mechanical

environment appears to be dependent on the FGF receptor and VEGF receptor signalling cascades and might be mediated by an additional cross-talk with other pathways. Recent evidence has shown that MSC transplantation significantly increases arteriogenesis coupled with recovery of cardiac performance [59]. Kinnaird *et al.* have demonstrated that marrow-derived stromal cells express genes encoding a broad spectrum of arteriogenic cytokines and promote *in vitro* and *in vivo* arteriogenesis through paracrine mechanisms [38].They demonstrated that MSCs augmented collateral remodelling through release of several cytokines such as VEGF and bFGF rather than *via* cell incorporation into new or remodelling vessels. They also found that murine with induced hindlimb ischemia when treated with MSC-conditioned media, showed an enhancement in collateral flow recovery and remodelling, and limb function compared with control media. Their findings imply that paracrine signalling is an important mediator of MSC therapy in tissue ischemia, and that cell incorporation into vessels is not a prerequisite for their effects.

Neovascularization induced by paracrine effects of MSCs is a complex process, which may comprise many molecules *via* intricate signal pathways. Several molecular mechanisms, such as SDF-1-mediated activation of eNOS, HGF-mediated calcineurin pathway, IGF-1-induced activation of PI3K and glycogen synthase kinase 3β dependent pathway have been revealed to participate in the process of neovascularization in experimental MI animals treated with MSCs [31, 32, 34, 60]. Furthermore, FGF and VEGF with their receptor signalling cascades coupled with cross-talk with other molecular pathways seem to be underlying mechanisms involved in the paracrine stimulation of neovascularization by MSC therapy [38, 58]. Numerous studies in the setting of MI have also shown that up-regulated expressions of these putative molecules by either genetic modification or preconditioning can enhance beneficial paracrine effects of MSC therapy. Growth factors and cytokines produced by MSCs may be used as potential strategy in cardiac repair after MI. However, interest should be generated to ascertain these putative molecular pathways prior to their use in future studies. In addition, whether paracrine factors produced by MSCs contribute to in-stent restenosis or atherosclerosis needs further assessment.

Anti-inflammatory effect of MSCs

It has been demonstrated that MSCs can exert paracrine cardioprotection effects in the surviving myocardium *via* regulation of large amounts of anti-inflammatory and pro-inflammatory factors. There is increasing evidence that MSC transplantation may decrease the high levels of protein production and gene expression of inflammatory cytokines TNF-α, IL-1β and IL-6, and others in response to injury, possibly by secreting a variety of anti-inflammatory cytokines, as well as changing the profile of cytokines released by immune cells. It is postulated that MSCs produce elevated prostaglandin E2 (PGE2), IL-4 and IL-10, which may act directly to limit deleterious, sustained endogenous inflammation in the heart. Aggarwal and Pittenger have observed that human MSCs co-cultured with purified subpopulations of immune cells alter the cytokine secretion profile of immune cells to induce a more anti-inflammatory or tolerant phenotype *in vitro* [61]. This study suggested that MSCs induced immune cells to decrease the secretion of TNF-α and interferon γ, whereas to increase the secretion of IL-10 and IL-4. In addition, MSCs produced elevated PGE2 in co-cultures, and inhibitors of PGE2 production mitigated MSC-mediated immune modulation. Another study has revealed that IL-10 may be partly responsible for the therapeutic efficacy of MSCs in the infarcted myocardium possibly *via* the IL-10-mediated differentiation of regulatory T cell [62]. MSC transplantation attenuates the cytotoxic activity of spleen lymphocytes and inhibits the activity of NF-κB, attenuates the protein production of TNF-α and IL-6, and increases the expression of IL-10 in infarcted myocardium [63]. MSCs may release heme oxygenase-1 (HO-1), an important anti-oxidative stress and graft survival protein in cardiac ischemic injury, resulting in protection in both transplanted MSCs and surviving CMCs, and improvement in cardiac function during the early stage after MI [64]. The beneficial effects of HO-1 in MSC-therapy in the MI setting may be associated with the reduction in the expression of TNF-α, IL-1-β and IL-6 mRNA and the increase in the expression of IL-10 mRNA, accompanied by increases of the expression of bFGF and VEGF [65]. It has recently been reported that it is the TNF receptor (TNFR)2, but not TNFR1 that enhances MSC-mediated cardiac protection following acute ischemia [66]. The authors concluded that TNFR2 likely mediates beneficial effects in MSCs, whereas TNFR1 signalling may damage MSC paracrine effects and decrease MSC-mediated cardioprotection. The adverse effects of TNFR1 signalling in MSC paracrine effects may be associated with increases in TNF and IL-6 expression, accompanied by decrease in VEGF expression [67]. It is postulated that TNFR2 mediates beneficial paracrine effects of MSCs likely *via* regulating these cytokines in a converse manner. How MSCs alter immune cells to induce a more anti-inflammatory phenotype after MSC transplantation in infarcted myocardium warrants further research.

Anti-apoptotic effects by MSCs

Accumulating evidence suggests that improved cardiac function is partly associated with inhibition of apoptosis provided by cytokines released from stem cells following transplantation. Transplantation of hypoxic cultured MSCs further enhances morphological and functional benefits in infarcted hearts, in part due to increases of Bcl-2 and its receptor Bcl-xL, which has been shown to prevent cell death and apoptosis under hypoxic conditions [68, 69]. Akt may significantly enhance retention of MSCs engraftment within infarcted myocardium and alter the secretion of various cytokines and growth factors [41]. This study further confirms that early paracrine mechanisms mediated by MSCs are responsible for enhancing the survival of existing myocytes. Conditioned medium from hypoxic Akt-modified MSCs (Akt-MSCs) markedly inhibits hypoxia-induced apoptosis and triggers vigorous spontaneous contraction of adult rat CMCs *in vitro*, and

significantly limits infarct size and improves cardiac performance in comparison with controls *in vivo* [42]. These protective effects are postulated to be associated with levels of growth factors, such as VEGF, FGF-2, HGF, IGF-I and thymosin β_4 (TB4). It has also been shown that Akt-MSCs promote CMC survival through paracrine mechanisms mediated by Sfrp2 *via* increasing cellular β-catenin and up-regulating expression of anti-apoptotic genes Birc1b and Bcl2, and others *in vivo* and *in vitro* [70]. Wang *et al.* have revealed that conditioned medium from MSCs engineered with heat-shock protein-20 protected adult rat CMCs against oxidative stress, possibly *via* enhanced activation of Akt and increased secretion of growth factors VEGF, FGF-2 and IGF-1 [71]. It has been indicated that MSC transplantation may have significant beneficial effects on injured heart function independent of cardiac regeneration and SDF-1 secreted by MSCs may play a role in trophic support for cardiac myocytes after MI [72]. The indirect evidence also comes from that pre-treatment of MSCs with a combination of FGF-2, IGF-1 and BMP-2, reduced apoptosis of neighbouring CMCs in a hypoxic condition and enhanced the phosphorylated Akt and phosphorylated cyclic adenosine monophosphate (cAMP) response element binding protein expression of CMCs, resulting in smaller infarct size and better cardiac function [73]. The pathways by which MSCs exert the property of anti-apoptosis are far from clear, possibly that cardiac regulation of Bcl-2/Bax ratio and activation of PI3K/Akt, mitogen-activated protein kinases pathways, including extracellular signal-regulated kinase (ERK), c-JUN N-terminal kinase (JNK) and p38, and activation of caspase-3 may be involved in this paracrine effect [74–77]. Thus, further detailed studies are required to provide exact molecular pathways leading to this protection.

Cardiac remodelling induced by paracrine effects

The remodelling of the left ventricle following MI represents a major cause of infarct-related heart failure and death. The majority of experiments have revealed that transplantation of MSCs can influence extracellular matrix remodelling through regulation of MMPs and matrix metalloproteinase endogenous inhibitor (TIMP) production and enhancing expression of anti-fibrotic factors, contributing to attenuation in cardiac remodelling after MI. MSCs may also exert paracrine anti-fibrotic effects to attenuate ventricular remodelling through regulation of cardiac fibroblasts (CFB) proliferation. It has been revealed that MSC-conditioned medium up-regulates anti-proliferation-related genes such as elastin, myocardin and DNA-damaged inducible transcript 3, and significantly down-regulates type I and III collagen expression and suppresses type III collagen promoter activity [78]. Mias *et al.* demonstrated that MSCs performed their anti-fibrotic properties to enhance MMP secretion by CFB and reduce cardiac ventricular fibrosis after MI [79]. They revealed that conditioned medium from MSCs decreased viability, α-smooth muscle actin expression and collagen secretion of CFB, concomitant with the stimulation of MMP-2/MMP-9 activities and membrane type 1 MMP expression, thus contributing to the improvement of morphological and functional cardiac parameters after intracardiac injection of MSCs in a rat model of post-ischemic heart failure. MSC transplantation can alter collagen dynamics and expression, and both the mRNA and protein expression of TIMP-1 and TGF-1β, resulting in decreased ventricular remodelling after MI [80]. A recent study in acute MI hearts, post-MSC transplantation has revealed that the ratio of TIMP-2 to MMP2, and TIMP-3 to MMP9 in MSC-grafted hearts was increased significantly in infarcted myocardium, and MSCs modified with HO-1 may further normalize the ratio of MMPs/TIMPs and attenuate remodelling [81]. Some cytokines, such as adrenomedullin (ADM), HGF, bFGF, IGF-1 and others, released from MSCs, possibly participate in the MSC-mediated attenuation of cardiac remodelling and contractile dysfunction in the infarcted heart following cell transplantation. MSC transplantation can suppress the function of CFB by secreting ADM, an anti-fibrotic factor, resulting in improvement of cardiac performance, partly through the decrease of myocardial fibrosis [82]. This study revealed that conditioned media, including ADM from cultured MSCs obviously inhibited CFB proliferation and expression of collagen I and III mRNA *in vitro*. *In vivo*, compared with medium transplantation, MSC transplantation significantly improved heart function, decreased collagen volume fraction and increased expression of ADM. Transplantation of MSCs engineered with the ADM gene can further enhance this beneficial effect after MI [83]. Nevertheless, more studies are required to interpret the molecular pathways by which MSCs exert their paracrine effects on altering CFB phenotype and collagen metabolism in the process of cardiac remodelling.

Paracrine-mediated cardiac contractility

There is growing evidence that MSCs may improve myocardial contractile performance after transplantation into the infarcted heart. MI significantly reduces fractional shortening, CMC peak shortening and maximal velocity of shortening and relengthening, as well as reduces resting intracellular Ca^{2+}, intracellular Ca^{2+} rise and decay rate, all of which may be attenuated or reconciled by MSC therapy [84]. Hearts in the MI animals developed severe contractile dyskinesis in the infarct zone and border zone, which may be significantly improved to active contraction by MSC transplantation [85]. The authors suggested that this observed beneficial effect likely resulted from paracrine repair mechanisms due to too small a cell engraftment to provide a structural contribution to the damaged heart. Boomsma and his colleagues have revealed that intravenously injected MSCs are able to home to viable myocardium and preserve contractility, likely through an MSC-mediated paracrine response since infarct morphology was unchanged and labelled cells observed at two weeks exhibited no change of characteristics in comparison with the injected MSCs [86]. It can be postulated that MSCs may rescue cardiac contractility through their capacity to enhance TGF-1β and decrease TNF-α and IL-1β expression after engraftation into the infarcted heart, since TGF-1β may block depression of *in vitro* cardiac myocyte

contractility induced by pro-inflammatory cytokines TNF-α and IL-1β [87]. Gnecchi *et al.* have demonstrated that conditioned medium from hypoxic Akt-MSCs markedly inhibits hypoxia-induced apoptosis and triggers vigorous spontaneous contraction of adult rat CMCs *in vitro* [42]. This study suggests that up-regulated factors VEGF, FGF-2, HGF, IGF-I and TB4 in conditioned medium from hypoxic Akt-MSCs are potential mediators of the effects through a paracrine manner. Recent evidence implies that paracrine-mediated cytoprotection, anti-remodelling and pro-angiogenesis effects may at times help to elucidate the efficacy. For example, Nguyen *et al.* observed that intracoronary injection of concentrated biologically active factors secreted by MSCs could achieve early protection of ischemic myocardium and improve cardiac repair and contractility, partly due to anti-apoptotic and anti-remodelling effects [88]. However, the pathways and the putative molecules involved in this mechanism are far from complete, and more studies are required to precisely ascertain this mechanism.

Cardiac metabolic modulation by MSCs

Abnormalities in myocardial energy metabolism may contribute to contractile dysfunction and the progressive worsening of ventricular function following MI. MSCs are characterized by metabolic flexibility and postulated to possess the potential to positively affect myocardial metabolism after administration into the MI heart. Hu and colleagues found that profound bioenergetic abnormalities characterized by decrease in ATP levels, phosphocreatine-to-ATP ratio and mitochondrial F(1)F(0)-ATPase in peri-infarcted myocardial regions may contribute to the transition from compensated left ventricular remodelling to CHF following MI [89]. Transplantation of MSCs can significantly improve these adverse changes in infarcted hearts [85]. This study documented that MSC transplantation into the border zone of infarcted hearts caused significant improvement in myocardial contractile performance and reduction in wall stress, ultimately contributing to significant bioenergetic improvement from paracrine repair mechanisms. However, Gnecchi *et al.* found that hearts treated with MSCs demonstrated a transient decrease in the ratio of phosphocreatine, a small but persistent fall in pH and persistent increase in glucose (as 2-deoxy-glucose) uptake rate similar to untreated infarcted hearts [90]. They found that treatment with Akt-MSCs spared phosphocreatine stores and significantly limited the increase in 2-deoxy-glucose uptake inversely related to functional recovery, leading to preservation of normal metabolism and pH in the surviving myocardium after MI. Therefore, Akt, an anti-apoptotic gene, may be presumed to lead to the efficacy, at least partially, *via* its influence on cellular survival and viability of engrafted MSCs. To date, relatively few studies have considered cardiac metabolism mediated by MSC paracrine effects, and pathways and molecules involved in this mechanism remain unknown.

Other potential effects

Cardiac nerve sprouting

Several studies have revealed that, like their capabilities in neovascularization, transplantation of MCSs can induce cardiac nerve sprouting, resulting in the improved cardiac performance. Pak *et al.* have found that MSCs injected with bone marrow into swine infarcted hearts led to overexpression of cardiac tenascin, increased the magnitude of cardiac nerve sprouting in both atria and ventricles, and increased the magnitude of atrial sympathetic hyperinnervation [91]. Using antibodies opposed to growth-associated protein 43 (GAP43), tyrosine hydroxylase (TH) and three subtypes of tenascin by immunocytochemical staining, they found more $GAP43^+$ nerves, higher TH^+ nerve densities and higher tenascin expression in the animals treated with MSCs in comparison with animals treated with culture media, and more $GAP43^+$ nerve in comparison with animals treated with fresh bone marrow. Another study has revealed that transplantation of human MSCs expresses higher mRNA of nerve growth factor-β with TH^+ sympathetic nerves, whereas lower mRNA of connexin43 with reduced gap junctions than sham controls [92]. Zhang and his colleagues have found that cAMP enabled MSCs to gain neural marker expressions with neuronal function, such as calcium increase in response to neuronal activators, dopamine, glutamate and potassium chloride [93]. However, only a few cells induced by cAMP responded to the three neuronal activators and further lack the neuronal morphology, suggesting that although cAMP is able to direct MSCs towards neural differentiation, they do not achieve terminal differentiation. Whether cardiac nerve sprouting would play a role in the cardiac repair after MSC transplantation, needs to be further ascertained by more confirmation in future studies.

Anti-arrhythmic potential

Unlike skeletal myoblasts, the association between MSC transplantation and arrhythmia is still controversial. Macia and Boyden have cited that stem cell therapy is pro-arrhythmic, but Ly and Nattel have contended that stem cell therapy carries no excessive pro-arrhythmic risk and may produce important anti-arrhythmic consequences if properly applied [94, 95]. MSC transplantation may be a cause of arrhythmias, possibly resulting from the tissue heterogeneity between MSCs and CMCs, as well as transdifferentiated CMC immaturity. Chang *et al.* have revealed pro-arrhythmic potential of MSC transplantation by co-culturing MSCs with neonatal rat ventricular myocytes in different ratios *in vitro* [96]. They found that conduction velocity was decreased in co-cultures compared with controls, and re-entrant arrhythmias were induced in 86% of co-cultures containing 10% and 20% MSCs but not in controls or co-cultures containing only 1% MSCs. This phenomenon probably results from the mechanism of increase in tissue heterogeneity resulting from electrical coupling of inexcitable MSCs with myocytes.

In contrast, several clinical studies have revealed that transplantation of MSCs is safe and feasible without apparent malignant arrhythmias [97, 98]. Mills *et al.* have shown that MSC-based therapy enhances electrical viability in rats with MI [99]. In their study, optical mapping revealed that MSC therapy preserved electrical viability and impulse propagation in the border zone, accompanied by a more homogeneous pattern and expressed connexin proteins in MSC engraftation, contributing to the reduction in arrhythmia inducibility, in comparison with skeletal myoblast implantation and saline infusion. Using programmed electrical stimulation technique, Wang *et al.* recently reported that MSC injection ameliorated the inducibility of ventricular arrhythmias after MI in rats, through significantly reducing inducible ventricular tachycardias, raising ventricular fibrillation threshold and prolonging ventricular effective refractory period and action potential duration, as well as shortening activation time in infarcted border zone of left ventricular epicardium compared with phosphate buffered solution (PBS)-treated hearts [100]. The crucial step to pave the way to anti-arrhythmic therapy of MSCs after MI is to understand the intrinsic electrophysiological properties of MSCs, and track and modulate both physical and electrophysiological integration with cardiac tissue. In addition, the long-term arrhythmic potential of MSC-derived CMCs should be assessed in future studies. Indeed, as expressed by Ly and Nattel, MSC-based therapeutics may one day be more effectively anti-arrhythmic than presently available anti-arrhythmic drug therapy [95].

Conclusion

This review provides systemic insight into the mechanisms of MSC-based therapy for the injured heart following MI. Accumulating evidence from animal and preliminary human studies has confirmed that transdifferentiation does occur, however, the contractive function of CMCs is still under debate. Recent evidence indicates that the frequency of cell transdifferentiation is too small to repopulate the dead cells following MI. However, this inefficient process can be enhanced by methods, such as preconditioning and genetic modification. These methods represent important advancements because they may overcome the challenge of the issue of cell survival within the host microenvironment following MI, and further enhance proliferative capacity and production of growth factors and cytokines. Producing significant amounts of paracrine factors may be one of the greatest attributes of MSCs in cardiac repair after MI. It would then be feasible to use MSC-derived paracrine factors as a therapy strategy for myocardial recovery after MI. Genetically modified MSCs over-expressing factors or receptors may further enhance secretion of needed paracrine factors and seem promising for myocardial therapy. However, adequate clinical data of genetically modified MSCs for cardiac therapy after MI are lacking, therefore further investigations are necessary to ascertain their potential benefit and safety issues for patients suffering from MI. In addition, MSC exertion of paracrine effects in cardiac repair may depend on a cluster of factors rather than a single factor, and which factor may play the greatest role is still unknown, thus the systematic consequences and interlinked molecular pathways involved in the recovery of cardiac function must be considered further, prior to clinical practice. Whether cardiac nerve sprouting and anti-arrhythmic potential mediated by MSCs may at times contribute to the recovery of the injured heart and the low frequency of mortality in infarcted animals, are debatable and need further elucidation. It may be the combination of the transdifferentiation, cell fusion, paracrine effects and other potential attributes rather than its component exhibited by MSCs that contributes to the beneficial effects of MSC therapy for MI. Therefore, in order to maximize the efficacy of MSC therapy after MI, it is necessary to understand the genomic and proteomic substrates that regulate molecular signal pathways involved in the course of cardiac repair occurring after MSC transplantation in the host. Although MSC-based therapy holds promise in the future treatment of MI with resultant CHF, it may only be considered as an adjunctive therapy at present. Additionally, there is no standard consensus existing for culture procedures in clinical trials. Various questions still remain, such as which patients are candidates for this therapy, how many cells and when and which route to deliver these cells. Because the patient population of chronic ischemic heart disease after infarction is markedly different from the acute MI population, the methods for determining effective myocardial stem cell therapy and therapeutic procedures for the two populations are still challenging problems. Furthermore, the impact of co-morbid conditions, such as diabetes, hypertension, smoking and age, needs to be assessed carefully prior to wide clinical application. Optimal answers to all of these problems may pave the way for future research in the field of regenerative cardiology.

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Conflict of interest

The authors confirm that there are no conflicts of interest.

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