

# Bone marrow mesenchymal stem cells for post-myocardial infarction cardiac repair: microRNAs as novel regulators

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## Abstract

Transplantation of bone marrow-derived mesenchymal stem cells (MSCs) is safe and may improve cardiac function and structural remodeling in patients following myocardial infarction (MI). Cardiovascular cell differentiation and paracrine effects to promote endogenous cardiac regeneration, neovascularization, anti-inflammation, anti-apoptosis, anti-remodelling and cardiac contractility, may contribute to MSC-based cardiac repair following MI. However, current evidence indicates that the efficacy of MSC transplantation was unsatisfactory, due to the poor viability and massive death of the engrafted MSCs in the infarcted myocardium. MicroRNAs are short endogenous, conserved, non-coding RNAs and important regulators involved in numerous facets of cardiac pathophysiologic processes. There is an obvious involvement of microRNAs in almost every facet of putative repair mechanisms of MSC-based therapy in MI, such as stem cell differentiation, neovascularization, apoptosis, cardiac remodeling, cardiac contractility and arrhythmias, and others. It is proposed that therapeutic modulation of individual cardiovascular microRNA of MSCs, either mimicking or antagonizing microRNA actions, will hopefully enhance MSC therapeutic efficacy. In addition, MSCs may be manipulated to enhance functional microRNA expression or to inhibit aberrant microRNA levels in a paracrine manner. We hypothesize that microRNAs may be used as novel regulators in MSC-based therapy in MI and MSC transplantation by microRNA regulation may represent promising therapeutic strategy for MI patients in the future.

**Keywords:** microRNA • mesenchymal stem cell • myocardial infarction • cardiac repair

## Introduction

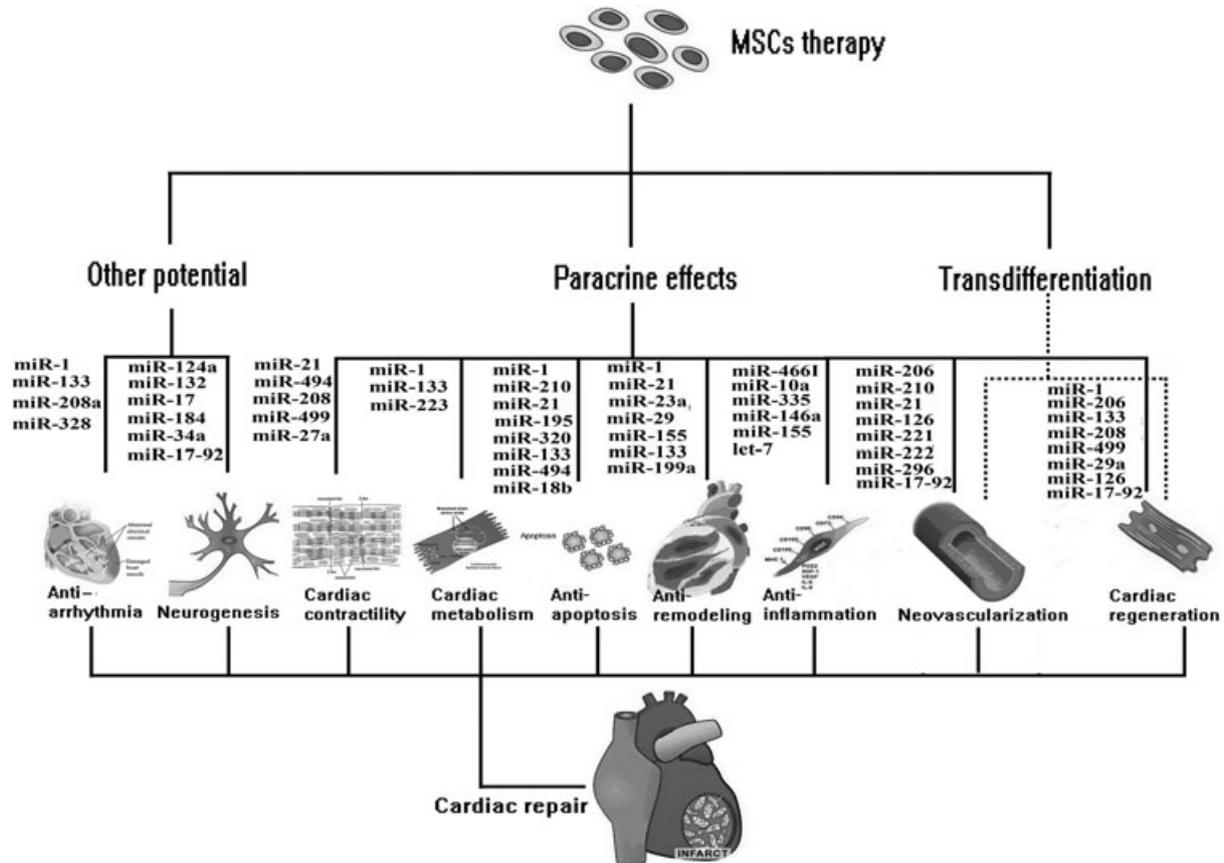
Myocardial infarction (MI) with resultant congestive heart failure (CHF) is a leading cause of mortality and morbidity in developed countries. Despite recent improvements in disease prevention and combinational therapy (medical, interventional, device and transplantation) for MI and CHF, the one-year mortality rate for patients with acute MI and impaired left ventricular (LV) function still ranges toward 13% [1]. The emergence of stem cell-based and microRNA (miRNA, miR)-based therapeutic strategies may repre-

sent a promising outlook for patients with cardiovascular disease (CVD), especially in the setting of MI.

MiRNAs, a large family of post-transcriptional regulators of gene expression, are approximately 22 nucleotides in length, and control many developmental and cellular processes in eukaryotic organisms. MiRNA expression is tightly controlled in a tissue-specific and developmental stage-specific manner, and some of these miRNAs are highly and specifically expressed in cardiovascular

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**Fig. 1** Summary of putative microRNAs that may be used as important modulators in mesenchymal stem cell (MSC)-mediated cardiac repair processes in myocardial infarction. These microRNAs may play central roles in numerous cardiac pathophysiologic processes, such as stem cell differentiation, cardiovascular cell proliferation and migration, myocardial inflammation and apoptosis, cardiac remodelling, contractility, metabolism and arrhythmias, and others. Preconditioning MSCs by microRNA modulation may enhance cell capacity to differentiate into cardiovascular cells, enhance anti-arrhythmic and cardiac nerve sprouting potential, and release more paracrine factors and functional microRNAs in a paracrine/autocrine manner, leading to better cardiac repair (see the text for details).

tissues [2]. There is increasing evidence revealing that signature expression patterns of miRNAs play an important role in pathological cardiac hypertrophy, cardiac arrhythmia and MI in human beings and animal models of heart disease. Gain- and loss-of-function studies in animal models have revealed profound and unexpected functions for these miRNAs in numerous facets of cardiac biology, including the regulation of stem cell differentiation, cardiac regeneration, neovascularization, apoptosis, cardiac remodelling and cardiac contractility [2–4]. It has been shown that some miRNAs expressed in human heart are significantly dysregulated in acute MI patients in comparison with healthy controls, and some miRNAs in peripheral blood show the highest sensitivity and specificity for the discrimination of MI cases from controls [5, 6]. It is proposed that miRNAs should be used as novel regulators and potential therapeutic targets for MI with resultant CHF, due to their important regulatory roles involved in various physiological and pathological processes following MI, despite that their roles for diagnostic, prognostic and therapeutic applications in MI still need to be systematically evaluated [5, 7].

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, accompanied by their capacity to differentiate to osteoblasts, adipocytes and chondroblasts under standard differentiating conditions *in vitro* [8]. Increasing evidence suggests that they are a promising source for cell therapy in the setting of MI with subsequent CHF. We have observed that administration of MSCs can improve myocardial function in MI rat models [9, 10], and their beneficial effects on cardiac repair following MI depend on their capacity to differentiate into cardiomyocytes (CMCs) and vascular cells, their capacity to release paracrine factors and their potential anti-arrhythmic and cardiac nerve sprouting efficacies [11].

It is likely foreseen that miRNAs may play critical roles in many pathological cardiac processes following MI and may be considerable as a novel potential regulator in the MSC-based treatment for MI, such as cardiovascular cell differentiation, paracrine effects and anti-arrhythmic effects, *etc.* (Fig. 1). However, the role of miRNAs in the MSC-based therapy for MI is yet to be understood.

Basing on our previous review [11] that mainly focused on experimental studies and clinical trials with bone marrow MSCs, we herein review current knowledge of the roles of miRNAs in different biological and pathological processes involved in CVD, especially in MI. We attempt to provide evidence supporting that the pre-motory potential of miRNA targets may be used as a promising strategy for MSC-based therapy for MI.

## MiRNAs and MSC differentiation into cardiovascular cells

MI leads to a significant loss of cells and formation of scar tissue. The remaining CMCs and vascular cells are unable to reconstitute the necrotic tissue, and cardiac function deteriorates during the ensuing course. We have observed that MSCs can be induced to differentiate into CMCs, vascular smooth muscle cells (VSMCs) and endothelial cells (ECs) *in vitro*, and *in vivo* through different administrations, contributing to the generation of *de novo* myocardium and a network of capillaries and larger size blood vessels [11]. Global gene expression analysis has revealed that MSC differentiation into specific mature cell types is a temporally controlled and regulated process involving the activities of various transcription factors, growth factors and signalling pathways [12]. Growing studies have not only identified miRNAs indicative of MSC differentiation patterns, but also demonstrated that extracellular signals contribute to miRNA regulation during differentiation, supporting a role for miRNAs during MSC transplantation [13].

### MiRNAs and MSC differentiation into CMCs

Despite that the potential of direct transdifferentiation into CMCs is still under debate, CMC differentiation from engrafted MSCs may be one of the potential mechanisms involved in the process of cardiac repair following MI [11]. MiRNAs, such as miR-1, miR-133, miR-208 and miR-499, have been shown to play important roles in the differentiation from stem cells to CMCs [4]. For example, overexpression of miR-499 and miR-1 resulted in up-regulation of important cardiac myosin heavy-chain (MHC) genes in embryoid bodies, and miR-499 overexpression also caused up-regulation of the cardiac transcription factor *Mef2c* [14]. MiR-1, specifically expressed in cardiac precursor cells, accompanied by miR-133, has been revealed to exhibit directly transcriptional regulation by serum response factor (SRF) and *Mef2* accompanied by target *Hand2*, a transcription factor that promotes ventricular CMC expansion in the heart [15, 16]. These findings imply regulator roles of miRNAs in CMC differentiation from cardiomyogenic stem cells.

MiRNA differentiation signatures may be used as reliable molecular markers specific to MSCs [17]. The high expressed miRNAs in microvesicles which can be released from MSCs have been described as a new mechanism of cell-to-cell communication in CMC differentiation [18]. The mechanism involved in this

efficacy may seem to be that engrafted MSCs communicate with target CMCs through microvesicles or exosome mediated transfer of genetic information, including miRNAs [19]. MiRNAs, such as miR-17, miR-19b, miR-20a, miR-29c, miR-499 and *let-7f*, and others, dysregulated in cultured MSCs upon *in vitro* passage may be novel markers of cell aging [20, 21], which may further explain that MSCs from aged donors have a marked decrease in the overall expansion rate and multilineage differentiation potential and may contribute to better choices of donor cells.

Therefore, identification of miRNAs expressed in engrafted MSCs will lead to the discovery of important regulatory roles for these small RNAs during CMC differentiation through their involvement in cell-to-cell signals and other influential factors. Additionally, it has been demonstrated that several tumours directly derives from transformed MSCs, such in the case of Ewing tumours upon activation of the *EWS-FL11* oncogene, and malignant fibrous histiocytoma upon inactivation of the *Wnt* signalling pathway [17]. Cardiac-specific miR-1 repressed translation of *Met* and blocked the growth of rhabdomyosarcoma xenografts *in vivo* by promoting myogenic differentiation [15]. Albeit none of the malignant conversions derived from transformed MSCs have been reported in MSC-based therapy in MI models and human beings to date, complete understanding of the roles and molecular targets of miRNAs will help to avoid and prevent potential deleterious effects, such as unwanted differentiation from engrafted MSCs and tumourigenesis promoted by MSC-derived CMCs.

### MiRNAs and MSC differentiation into vascular cells

Like their potential roles in CMC differentiation, miRNAs may also play essential roles in VSMC and EC differentiation derived from engrafted stem cells, including MSCs, following MI [4, 22]. For example, miR-1, miR-126, miR-210, miR-145 and miR-143, and others, may be important mediators for EC and VSMC differentiation [23–26]. Current evidence has revealed that in mouse models of MI, systemic administration of an antagomiR designed to inhibit miR-92a targeting several proangiogenic proteins, including the integrin subunit  $\alpha$ -5, led to enhanced blood vessel growth and functional recovery of damaged tissue, suggesting that miRNAs may serve as a valuable therapeutic target in the setting of MI [27].

It has been shown that transplanted MSCs are preferentially attracted to the infarcted, but not the noninfarcted, myocardium [28]. Accordingly, the expression signature of miRNAs is found to be different and aberrant in the infarcted areas and border areas in comparison with the noninfarcted areas [29]. Microvesicles, a new mechanism of cell-to-cell communication, including numerous miRNAs from injured vascular cells may reprogram the phenotype of stem cells to acquire specific features of the tissue through transfer of genetic information between stem cells and injured tissue in a paracrine/endocrine mechanism [30]. For example, direct cell-to-cell communication between MSCs and angioblasts has been proved to play a pivotal role in the differentiation of MSCs to ECs and VSMCs [31]. It is proposed that change of some miRNAs

**Table 1** Bone marrow mesenchymal stem cells-secreted paracrine factors in cardiac repair mechanisms

Mechanisms of action	Putative paracrine mediators
Cardiac regeneration	VEGF, IGF-1, HGF, BMP-2, TGF- $\beta$ , SDF-1, FGF-2, Sfrp2
Neovascularization	VEGF, IGF-1, HGF, bFGF, PDGF-BB, PGF, Ang-1, TGF- $\beta$ , SDF-1, MMP-2, MMP-9
Anti-inflammation	IL-4, IL-10, PGE2, HIF-1 $\alpha$ , TGF- $\beta$ , TSP-1, IL-6
Anti-apoptosis	VEGF, IGF-1, HGF, bFGF, TNF- $\alpha$ , SDF-1, TB-4, Sfrp2, EPO, FGF-2, ADM, BMP-2
Anti-remodelling	MMP-2, MMP-9, TIMP-1, TIMP-2, TIMP-9, IL-10, TB-4, TGF- $\beta$ , HGF, Tenacin C, SDF-1, ADM, IGF-1,
Cardiac contractility	VEGF, HGF, bFGF, TB-4, FGF2, IGF-1, TGF- $\beta$
Cardiac metabolism	HIF-1 $\alpha$ , IGF-1

VEGF: vascular endothelial growth factor; FGF: fibroblast growth factor; HGF: hepatocyte growth factor; IGF: insulin growth factor; Ang: angiotensin; 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  $\beta$ -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.

governing the production of growth factors, such as vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), insulin-like growth factor-1 (IGF-1) and stromal cell-derived factor-1 $\alpha$  (SDF-1 $\alpha$ ) and others, in the hypoxic conditions, may influence the capacity of MSC differentiation into vascular cells. SDF-1 $\alpha$ , an important mediator of stem cells attracted to the injured heart, can directly induce MSC differentiation into ECs *in vitro* [32], while MSCs engraftment was blocked by pretreatment with miRNA targeting C-X-C chemokine receptor (CXCR) 4, leading to reverse benefits associated with significantly decreased angiogenesis by vascular cell differentiation in the peri- and infarcted areas of the heart [33]. MiRNA expression profiling in MSCs are associated with donor age which has been proved to be associated with the vascular cell differentiation efficacy of MSCs in host infarcted heart [34, 35], representing novel insights into the aging process and the potential for autologous MSC therapy in older MI patients. Detailed identification of roles of miRNAs will maximize the efficacy of angiogenesis by MSC differentiation into angioblasts after MI and further assess the association between MSC-derived vascular cells and potential risks for adverse effects, such as in-stent restenosis and atherosclerosis.

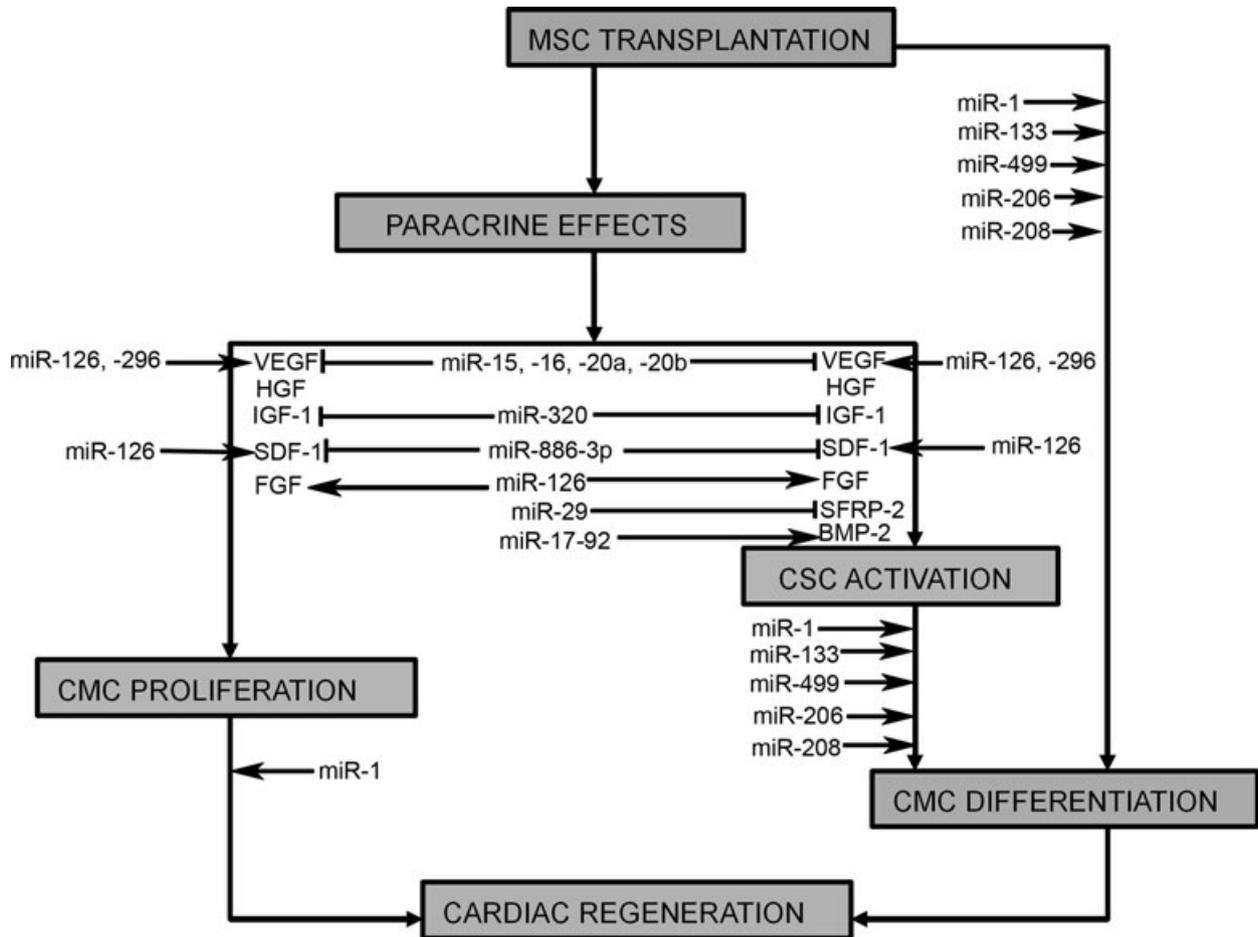
## MiRNAs and MSC-mediated paracrine effects

MSCs can secrete growth factors and cytokines which can exert their influence on cardiac repair in a paracrine fashion (Table 1) [11]. It has been suggested that the expression of more than 30% of protein-coding genes can be regulated by miRNAs in human beings [12]. In addition, MSCs can potentially exert miRNA-

mediated biological effects on target cells through the secretion of miRNAs and pre-miRNAs in microvesicles, thus contributing to down-regulating specific targets in target cells [30, 36]. Microvesicles/exosomes have been described as a potent paracrine mechanism that may redirect cell fate through the active transfer of functional miRNAs, and microvesicles may deliver to target cells not only endogenous miRNAs, but also traceable synthetic miRNAs [18], which makes modulation of miRNAs in MSCs prior to transplantation more feasible.

## MiRNAs and MSC-mediated endogenous cardiac regeneration

Myocardial regeneration subsequent to MI could also be accomplished by alternatively stimulating the endogenous stem cells and surviving CMCs to more aggressively participate in the repair process in a paracrine manner, which may be regulated by miRNAs (Fig. 2). The heart has an endogenous reserve of cardiac stem cells (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 [11]. Post-transcriptional regulation of gene expression, mediated by miRNAs, may play an essential role in the control of CMC differentiation [37]. Sluijter *et al.* [38] found that transient transfection of muscle-specific miR-1 and miR-499 in CMC progenitor cells enhanced differentiation into CMCs in human CMC progenitor cells and embryonic stem cells, likely *via* the repression of histone deacetylase 4 or Sox6. Bone morphogenetic protein 2 (BMP-2) regulates myocardial differentiation *via* regulation of the miRNA-17-92 cluster, seeding sequences within the 3' untranslated regions (UTR) of cardiac progenitor genes such as *Isl1* and *Tbx1*, contributing to down-regulation of cardiac progenitor genes



**Fig. 2** Proposed microRNAs may be used as important modulators involved in mesenchymal stem cell (MSC)-mediated cardiac regeneration. Potentially, these microRNAs may be manipulated in engrafted MSCs to enhance their efficacy to differentiate into cardiomyocytes (CMCs), release more functional paracrine factors to induce activation, migration, and differentiation of cardiac stem cells (CSCs) and enhance proliferation of cardiomyocytes residing in the heart. The positive activation is indicated by arrows while the negative activation is shown by lines with blocked ends (see text for details). VEGF: vascular endothelial growth factor; FGF: fibroblast growth factor; HGF: hepatocyte growth factor; IGF-1: insulin growth factor; SDF: stromal cell-derived factor; Sfrp: secreted frizzled related protein; BMP: bone morphogenetic protein.

and enhancement in myocardial differentiation [39]. Secreted frizzled related protein 2 (Sfrp2), a member in the Wnt signalling and direct target of miR-29a [40], has also been shown to regulate cardiomyogenic differentiation by inhibiting a positive transcriptional autoregulation loop of Wnt3a [41]. Thus, modulation of miR-17-92 and miR-29a to regulate the myocardial differentiation signalling in MSCs may promote more endogenous myocardium accompanied by avoidance of undesired differentiation in the infarcted heart following MSC transplantation. MiR-886-3p and miR-126 may be potential target miRNAs to be preconditioned in MSCs to increase the expression of SDF-1 prior to engraftment, contributing to enhance SDF-1 chemoattractive efficacy to recruit more stem cells and progenitor cells into the infarcted heart [42, 43].

Endogenous cardiac regeneration from resident CSCs and CMCs may avoid unwanted differentiation and malignant prolifer-

ation, coupled with other potential side effects. Therefore, the use of conditioned medium of miRNA-preconditioned MSCs rather than MSCs alone may be a viable option for efficient cardiogenesis in future research. However, more direct research is required to precisely ascertain the role of miRNAs in the course of endogenous cardiac regeneration occurring after MSC transplantation in the host myocardium.

### MiRNAs and MSC-mediated cardiac contractility

MI significantly leads to severe contractile dyskinesia in the infarcted zone and border zone. Growing evidence has demonstrated that up-regulated factors VEGF, FGF-2, HGF and IGF-1 in conditioned medium from hypoxic Akt-modified-MSCs markedly

inhibits hypoxia-induced apoptosis and triggers vigorous spontaneous contraction of adult rat CMCs *in vitro* through a paracrine manner, and changes of miR-21 and miR-494 are required partly for the activation of Akt-mitochondrial signalling pathway [44–46]. Patrick *et al.* [47] have shown that miR-21-null mice are normal and, in response to a variety of cardiac stresses, display up-regulation of stress-responsive cardiac genes and loss of cardiac contractility comparable to wild-type littermates.  $\alpha$ -MHC and  $\beta$ -MHC, the primary contractile proteins of the heart, are important modulators for diminution of CMC contractility in response to stress of myocardial ischaemia, especially in MI. MiR-208 can regulate the  $\alpha$ -MHC gene to encode a major cardiac contractile protein, regulate cardiac growth and gene expression in response to stress and hormonal signalling [48]. Cardiac knockout mice of miR-208a are normal at birth, but show a slight reduction in contractility as measured by fractional shortening at 2 months of age [37]. MiR-27a has also been shown to regulate cardiac contractile protein  $\beta$ -MHC which regulates cardiac contractility [49]. These findings indicate that the miRNA family is an important modulator of cardiac contractility and may be used as a novel modulator of MSC-mediated cardiac contractility preservation after transplantation into the infarcted heart. However, the pathways and the putative molecules involved in this mechanism are far from complete, and the roles of miRNAs in this process still need to be precisely ascertained by more in-depth studies.

## MiRNAs and MSC-mediated neovascularization

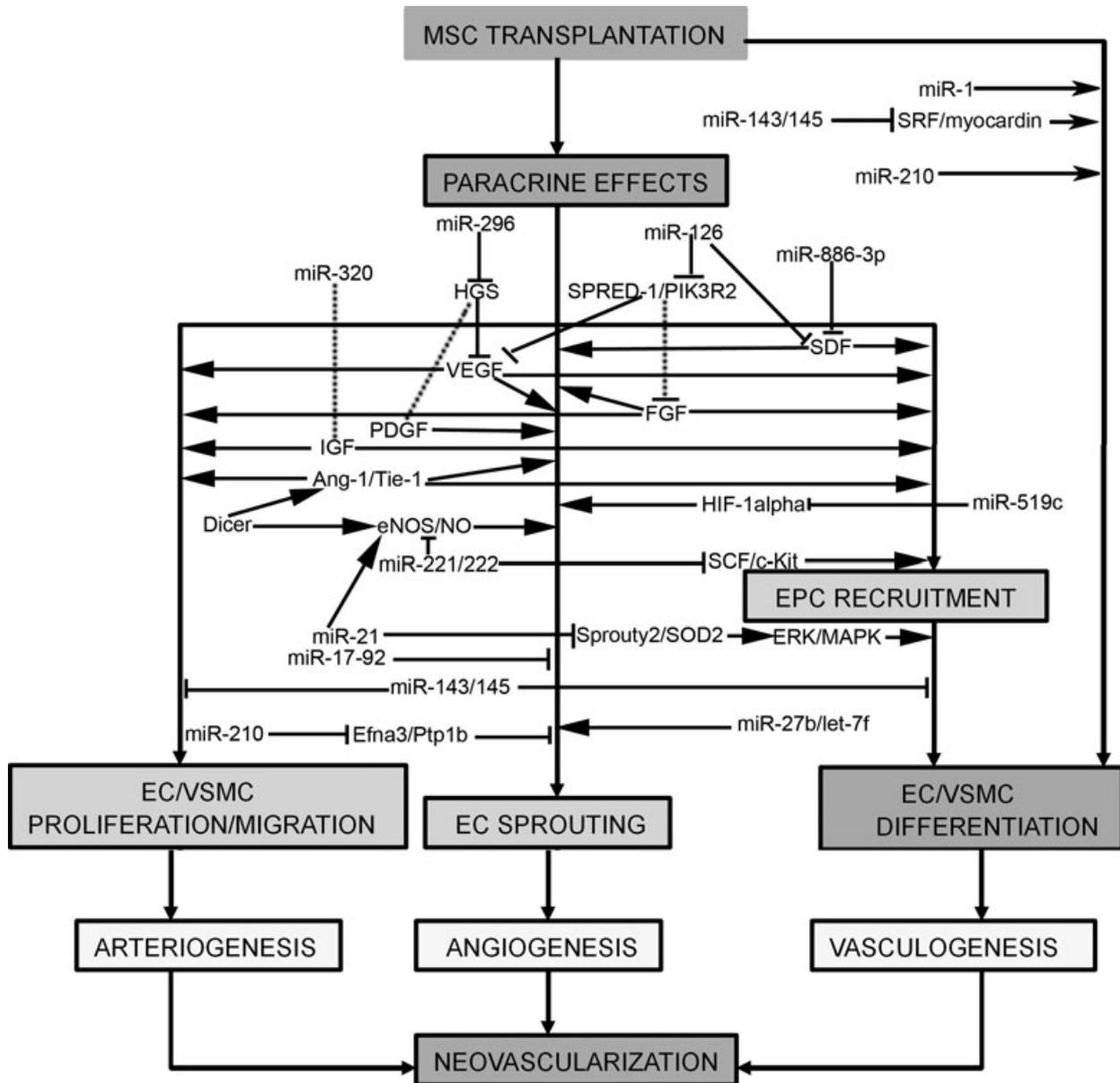
Neovascularization, including vasculogenesis, angiogenesis and arteriogenesis, is another important biological process positively influenced by engrafted MSCs in cardiac repair after MI. Numerous paracrine factors released from engrafted MSCs (Table 1), accompanied by several molecular mechanisms, including SDF-1-mediated activation of endothelial nitric oxide synthase (eNOS), FGF receptor and VEGF receptor signalling cascades, IGF-1-induced activation of PI3K and glycogen synthase kinase 3  $\beta$ -dependent pathways have been shown to participate in the process of neovascularization in experimental MI animals treated with MSCs [11]. Growing evidence has identified that miRNAs can play central roles in various aspects of neovascularization, such as differentiation, proliferation, migration, and morphogenesis of vascular cells (Fig. 3), through targeting these putative paracrine factors and molecular pathways *via* regulating gene expression at the post-transcriptional level [3, 50, 51]. The knockdown of Dicer, a key enzyme involved in the maturation of miRNAs, in ECs, altered the expression of several key regulators of endothelial biology and angiogenesis, such as VEGF receptor 2 KDR, angiopoietin receptors Tie-2 and Tie-1, eNOS and interleukin (IL)-8, and miR-222/221 were found to regulate eNOS protein levels after Dicer silencing by using miRNA mimicry [52]. In addition to its efficacy in vascular cell differentiation, miR-126 regulates the response of ECs to angiogenic growth factors VEGF and bFGF, in part by directly repressing negative regulators of these factor pathways, including the Sprouty-related protein SPRED1 and PI3K regulatory

subunit 2 (PIK3R2) [53]. MiR-126 has also been shown to participate in the process of angiogenesis by EC sprouting in response to blood flow [54]. The expression of these factors can also be regulated by other miRNAs, for example, miR-296 can up-regulate the production of VEGF and platelet-derived growth factor (PDGF) by directly targeting the hepatocyte growth factor-regulated tyrosine kinase substrate (HGS) mRNA [55]. However, due to competitive principle, miR-886-3p, if overexpressed, can impair MSC's efficacy to release SDF-1 *via* specifically targeting the 3' UTR of SDF-1 mRNA, resulting in the loss of SDF-1-directed chemotaxis for stem cell [42]. Similarly, the inhibition of miR-320 to significantly increase the expression of IGF-1 protein and the overexpression of miR-21 to increase eNOS phosphorylation and nitric oxide production may be a therapeutic strategy for the treatment of impaired angiogenesis in MI [56, 57]. However, miR-21 may impair angiogenic progenitor cells (APCs) function through gene repression of sprouty-2 and superoxide dismutase 2, leading to extracellular signal-regulated kinase (ERK) mitogen-activated-protein-kinase (MAPK)-dependent reactive oxygen species formation, and miR-21 blockade may rescue APC dysfunction in patients with coronary artery disease (CAD) [58]. In addition to their modulation in the production of eNOS protein, miR-221 and miR-222 have also been shown to impair stem cell factor (SCF) induced angiogenesis *via* targeting its receptor c-Kit [59].

Deciphering the miRNA network responsible for the fine-tuning of the process of neovascularization might lead to new therapeutic approaches to modulate neovascularization in MSC-based therapy in ischaemic conditions such as MI. In addition, the identification of the roles of miRNAs in the process of neovascularization may further assess the association between underlying adverse effects, such as in-stent restenosis or atherosclerosis, and MSC-mediated paracrine effects.

## MiRNAs and MSC-mediated anti-inflammatory effect

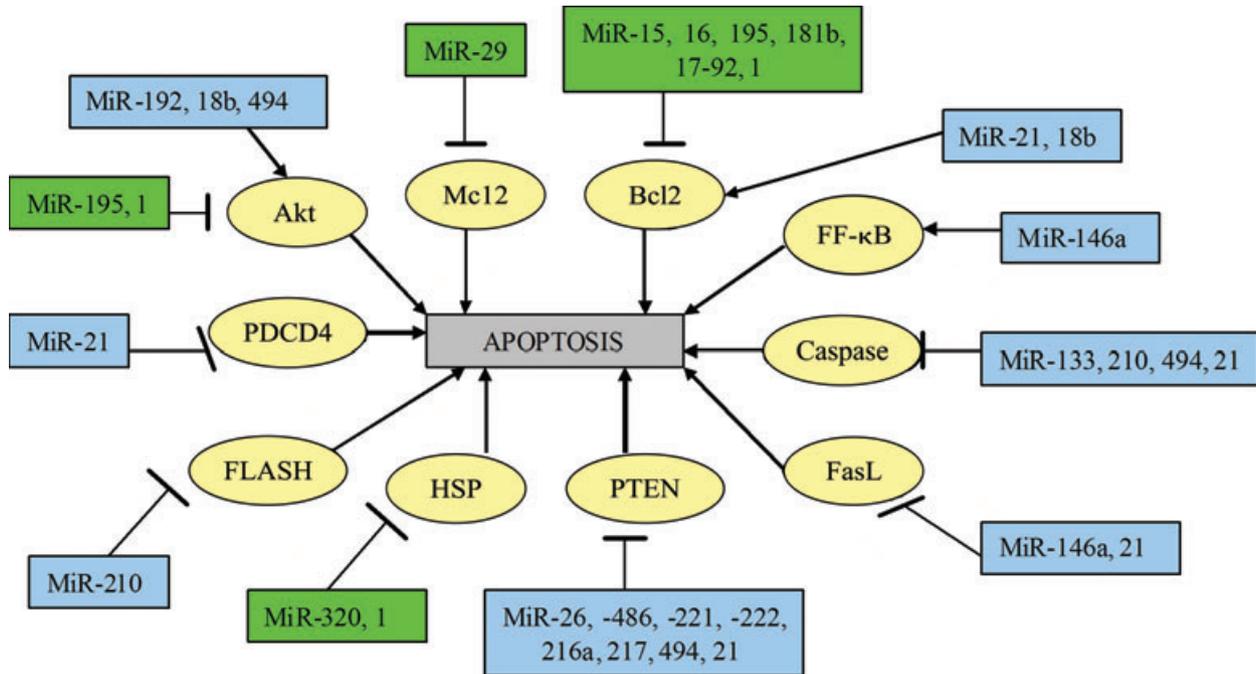
MSC can alter the cytokine secretion profile of immune cells to induce a more anti-inflammatory or tolerant phenotype, thus decreasing the secretion of TNF- $\alpha$ , IL-1 $\beta$ , IL-6 and interferon- $\gamma$ , but increasing the secretion of IL-10, IL-4 and prostaglandin E2 (PGE2), resulting in cardioprotection [11]. Accumulated evidence implies that the signalling of the innate immune response may be involved in myocardial adaptation to ischaemia following MI, and miRNAs play an essential role in the development and function of immune cells, especially in immune regulatory T cells [60]. MiR-466l up-regulates IL-10 expression in Toll-Like Receptor-triggered macrophages by antagonizing RNA-binding protein tristetraprolin-mediated IL-10 mRNA degradation, and IL-10-mediated differentiation of regulatory T cells has been shown to be partly responsible for the therapeutic efficacy of MSCs in the infarcted myocardium [61, 62]. Interferon (IFN)- $\gamma$ , a pro-inflammatory cytokine that plays an important role in activating the immunomodulatory properties of MSCs, can down-regulate the expression of miR-335 [63]. MSCs were shown to release



**Fig. 3** Proposed microRNAs involved in mesenchymal stem cell (MSC)-mediated neovascularization. MicroRNAs are important modulators in various aspects of neovascularization, thus can be manipulated in engrafted MSCs to directly differentiate into and indirectly induce endothelial progenitor cells (EPCs) to differentiate into endothelial cells (ECs) and vascular smooth muscle cells (VSMCs), induce EC sprouting from pre-existing vessels and induce EC/VSMC proliferation, migration and maturation, leading to vasculogenesis, angiogenesis and arteriogenesis, respectively. MicroRNAs exert these modulatory roles by targeting putative paracrine factors and molecular pathways that govern the process of neovascularization *via* regulating gene expression and through the active transfer of functional microRNAs between engrafted MSCs and target cells. The positive activation is indicated by arrows while the negative activation is shown by lines with blocked ends. VEGF: vascular endothelial growth factor; FGF: fibroblast growth factor; IGF: insulin growth factor; Ang: angiotensin; PDGF: platelet-derived growth factor; SDF: stromal cell-derived factor; HIF: hypoxia-inducible factor; SCF: stem cell factor; eNOS: endothelial nitric oxide synthase; NO: nitric oxide; SRF: serum response factor; HGS: hepatocyte growth factor-regulated tyrosine kinase substrate; PIK3R: phosphoinositide-3 kinase regulatory subunit; ERK: extracellular signal-regulated kinase; MAPK: mitogen-activated-protein kinase; SOD: superoxide dismutase.

microvesicles shuttling selected miRNAs and a few selected miRNAs shuttled by microvesicles were also associated with the immune system regulation, including leukocyte activation and dif-

ferentiation and haemopoiesis regulation [18]. Another study has even revealed that nuclear factor (NF)-kappaB can directly activate Lin28 transcription and rapidly reduce let-7 miRNA levels in breast



**Fig. 4** Proposed microRNAs may be used as important regulators in mesenchymal stem cell (MSC)-mediated anti-apoptotic effects. MicroRNAs shown in blue and green boxes are in general anti-apoptotic and pro-apoptotic, respectively. HSP: heat shock proteins; NF: nuclear factor; PDCD: programmed cell death; PTEN: phosphatase and tensin homologue deleted on chromosome 10; FasL: Fas ligand; FLASH: FLICE-associated huge protein.

cells, and Let-7 can directly inhibit IL-6 expression, resulting in higher levels of IL-6 [64]. Guo *et al.* [65] have showed that miR-146a treatment in peripheral blood mononuclear cells *in vitro* could induce the protein expression of pro-inflammatory cytokines tumour necrosis factor (TNF)- $\alpha$ , monocyte chemotactic protein-1 (MCP-1) and NF-kappaB p65 from Th1 cells, while miR-146a inhibitor could significantly attenuate these phenomena. MiR-10a may also significantly down-regulate inflammatory biomarkers MCP-1, IL-6, IL-8, vascular cell adhesion molecule 1 and E-selectin in atherosusceptible endothelium *in vivo* and *in vitro* [66].

Enhancing anti-inflammatory efficacy of MSCs by the miRNA regulator may support a novel strategy for MSC-based therapy post-transplantation in cardiac repair following MI. However, how MSCs alter immune cells to induce a more anti-inflammatory phenotype and how miRNAs modulate this process after MSC transplantation in infarcted myocardium, warrants further research.

### MiRNAs and MSC-mediated anti-apoptotic effect

Accumulating evidence suggests that improved cardiac function is partly associated with inhibition of apoptosis provided by cytokines released from stem cells following transplantation. Growing evidence has shown that miRNAs target numerous paracrine factors and proapoptotic and antiapoptotic signalling molecules involved in MSC-mediated anti-apoptotic effects in a paracrine manner (Fig. 4). For example, MiR-195 may be charac-

teristic of a proapoptotic regulator for its repression in multiple survival genes including the Bcl-2 gene family, the E2F gene family and Akt, while miR-18b may be characteristic of an antiapoptotic regulator for its potential to regulate multiple antiapoptotic genes, including Akt, Bcl-2, E2F and IGF-1 [67].

It has been revealed that miR-1 plays an important role in the regulation of CMC apoptosis in the ischaemia/reperfusion (I/R) rat model by targeting Bcl-2 [68]. Bcl-2 gene manipulated to overexpress in MSCs can prevent cell death and apoptosis under hypoxic conditions *in vitro* and further increase cellular survival at different time points *in vivo*, compared with the vector-MSC group [69]. AntagomiRs against miR-29a or miR-29c significantly reduced myocardial infarct size and apoptosis in hearts subjected to myocardial I/R injury, partly by targeting Mcl-2, an anti-apoptotic Bcl-2 family member [70]. Akt, an important anti-apoptotic signalling molecule, may significantly enhance retention of MSCs engraftment within infarcted myocardium and alter the secretion of various cytokines and growth factors. Hypoxia-induced down-regulation of miR-21 and up-regulation of its targets Fas ligand (FasL) and phosphatase and tensin homologue deleted on chromosome 10 (PTEN), in CMCs were reversed by activated Akt-dependent pathways [45]. The antiapoptotic function of Akt is partly through the inhibition of miR-21 in caspase-8 activity and mitochondrial damage. Another study has even revealed that miR-21 and pre-miR-21 ameliorate H(2)O(2)-induced cardiac cell death and apoptosis, possibly *via* programmed cell death 4 (PDCD4)/ activator protein 1 (AP-1) molecular signalling [71]. The

mechanism of Akt activation has also been revealed through the down-regulation of PTEN by miR-216a and miR-217 both regulated by upstream miR-192 and transforming growth factor (TGF)- $\beta$ , and the Akt activation by these miRNAs was similar to the effects of activation by TGF- $\beta$  [72].

MSCs modified with heat shock proteins (HSPs) genes or pretreated with HSPs, such as HSP70, HSP60 and HSP20, are protected from hypoxia-mediated cell death and rescue heart functions from myocardial injury [73, 74]. Furthermore, conditioned medium from MSCs engineered with HSP20 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 [73]. Ren and colleagues [75] have demonstrated that miR-320 is involved in the regulation of I/R-induced cardiac injury and dysfunction *via* antithetical regulation of HSP20. Furthermore, transgenic mice with cardiac-specific overexpression of miR-320 revealed an increased extent of apoptosis and infarct size in the hearts on I/R *in vivo* and *ex vivo* relative to the wild-type controls, which could be reversed by antagomiR-320 treatment. It is speculated that post-transcriptional repression of HSP60 and HSP70 by miR-1 and of caspase-9 by miR-133 may significantly contribute to their opposing actions in apoptotic cell death in MI [76]. MiR-1 and miR-206 have been observed to be involved in apoptotic cell death in MI by post-transcriptional repression of IGF-1 3'UTR of the position 175-196 in rats [77]. The production of HSP70 and heat shock factor 1 were reduced with increasing age in MSCs, accompanied by the up-regulation of miR-766 and miR-558 and the down-regulation of miR-let-7f, miR-125b, miR-222, miR-199-3p, miR-23a, and miR-221 [35], which may further account for impairment in the overall expansion rate and multilineage differentiation potential of MSCs from aged donors.

These studies indicate that miRNAs may be used as important regulators in MSC-mediated anti-apoptotic effects in MI treatment, due to their ability to regulate the expression levels of genes that govern the process of cell apoptosis. However, further direct studies are required to focus on the role of miRNAs involved in the process of anti-apoptosis in MSC-based therapy in MI.

### **MiRNAs and MSC-mediated anti-remodelling effect**

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 matrix metalloprotease (MMP) and matrix metalloproteinase endogenous inhibitor (TIMP) production and enhancing expression of anti-fibrotic factors, contributing to attenuation in cardiac remodelling post-MI [11]. Mesenchymal stem cells may also exert paracrine anti-fibrotic effects to attenuate ventricular remodelling through regulation of cardiac fibroblasts (CFBs) proliferation. Emerging evidence potentiates miRNAs to be important regulators in MSC-mediated anti-remodelling effects in MI treatment, due to their ability to regulate the expression levels of genes that govern the process of cardiac remodelling. MiRNAs may regulate key

components of the remodelling process, including CMC and CFB biology, cell fate, collagen metabolism and neurohormonal activation. Dicer and miRNAs, especially miR-188, were found to be involved in homocysteine-induced cardiac remodelling, attributing to regulate the expression of MMP-2, -9 and TIMP-1, -3, -4 [78]. Selectively increased levels of miR-21 in CFBs of the failing heart augment ERK-MAPK activity through inhibition of sprouty homologue 1, contributing to the extent of interstitial fibrosis and cardiac hypertrophy, while the silencing of miR-21 by a specific antagomiR can inverse these adverse effects and attenuate cardiac dysfunction [79]. Wang *et al.* [80] have revealed that changes in miR-23a and miR-29a were the most significant among 40 of 293 miRNAs changed >2-fold in the hearts from rats subjected to abdominal aortic constriction, indicating these miRNAs important roles in heart remodelling and reverse remodelling. Down-regulation of miR-29 with anti-miRNA *in vitro* and *in vivo* induces the expression of collagens, whereas overexpression of miR-29 in fibroblasts reduces collagen expression [81], which was similar to the other study revealing miR-29a coupled with miR-25 was sufficient to decrease collagen gene expression when transfected into isolated CFBs *in vitro* [82]. MiR-133 along with miR-30 directly down-regulates the connective tissue growth factor, a key profibrotic protein, accompanied by decreased production of collagens, leading to the amelioration of cardiac remodelling [83]. In addition, MSCs over-expressing CXCR4 could up-regulate MMP expression and alleviate early signs of LV remodelling, while MSCs pretreated with miRNA targeting CXCR4 may weaken this benefit [33].

These findings suggest that miRNAs possess potential to act as a regulator of cardiac remodelling and represent potential therapeutic targets for cardiac remodelling after MI. However, the role of miRNAs in cardiac remodelling following MI and its role in MSC-based therapy for MI are still far from clear.

### **MiRNAs and MSC-mediated cardiac metabolic effect**

Abnormalities in myocardial energy metabolism may contribute to contractile dysfunction and the progressive worsening of LV function following MI. MSCs are characterized by metabolic flexibility and postulated to possess the potential to positively reverse profound bioenergetic abnormalities in peri-infarcted myocardial regions after MI [11]. Adenosine kinase (ADK), the major adenosine-removing enzyme, may be regulated by miRNAs in MSCs to adapt its intra- and extra-cellular levels in response to environmental changes [84]. The authors concluded that lentiviral expression of anti-ADK miRNAs constituted a versatile tool to generate therapeutically effective adenosine releasing human MSCs, which may also be used in MSC-mediated cardiac metabolic modulation. Furthermore, Lu and colleagues [85] quantitatively analysed the expression of 155 miRNAs and found that miR-223 was consistently up-regulated in the insulin-resistant heart. In addition, siRNA knockdown of glucose transporter 4 (GLUT4) further ascertained that miR-223 overexpression-induced GLUT4 protein expression in CMCs was necessary and sufficient for increased

glucose uptake. IGF-1 can inhibit glucose-induced mitochondrial dysfunction, cytochrome-c release and apoptosis in the rat CMC cell line H9C2 cells, possibly *via* miR-1 modulation, for miR-1 mimics, but not mutant miR-1, can block these capacities of IGF-1 [86]. It is proposed that activation of paracrine factors and signalling molecules, for example, activation of ADK and IGF-1 *via* miRNA modulation in MSCs, may present similar efficacy. However, to date, relatively few studies have considered cardiac metabolism mediated by MSC paracrine effects, and the role of miRNAs in this mechanism remains unknown.

## MiRNAs and MSC-mediated other potential effects

### MiRNAs and MSC-related cardiac neurogenesis

It is proposed that, like their capabilities in neovascularization, transplantation of MSCs can induce cardiac nerve sprouting, resulting in the improved cardiac performance [11]. Maisel *et al.* [87] have shown that MSCs can convert into a neural stem cell (NSC)-like population with all major properties of NSCs, contributing to neurogenesis. The authors concluded that HIF-1 and miR-124a were important regulators involved in this process of conversion. The regulatory role of miR-124 in this conversion may attribute to its interaction with a conserved miR-124 binding site in the 3'-UTR of NeuroD1 and negative regulation of the expression of the proneural marker NeuroD1, a basic helix-loop-helix transcription factor for neuronal differentiation [88]. Dicer and miRNAs maintain the NSC phenotype and facilitate the complete differentiation in NSC self-renewal and neurogenesis [89], which further validate miRNA regulatory efficacy. Other miRNAs, such as miR-17, miR-132, miR-16, let-7a, miR-184, miR-34a, miR-19a and miR-20a, may be important regulators in MSC differentiation into NSC with resultant neurogenesis [90–93], with respect to their regulation of NSC differentiation. These findings further support that miRNAs are involved in the fine control of cell proliferation and differentiation during the MSC-induced neurogenesis after transplantation into host infarcted heart. The efficacy of MSCs converting into NSCs will contribute to neurogenesis in the infarcted heart following cell transplantation. However, whether cardiac nerve sprouting participates in the process of the cardiac repair after MSC transplantation and what the roles of miRNAs display in this process, need to be further ascertained by more confirmation in future studies.

### MiRNAs and MSC anti-arrhythmic potential

Current clinical studies and animal experiments have revealed that transplantation of MSCs is safe and feasible without apparent malignant arrhythmias, and if properly applied, may produce

important antiarrhythmic consequences. MSC-based therapy provides great hope for anti-arrhythmia treatment for its efficacy to ameliorate electrophysiological characteristics of myocardium after transplantation into the host infarcted heart [11]. Growing evidence has shown that the miRNA family is an important modulator in cardiac arrhythmia. MiRNAs may control cardiac excitability *via* regulating the expression of multiple ion channels involved in the regulation of cardiac conduction, repolarization, and automaticity [94]. The authors have made a summary that several ion channel genes GJA1/Cx43/IJ, KCNJ2/Kir2.1/IK1, KCNH2/HERG/IKr, KCNQ1/KvLQT1/IKs, KCNE1/minK/IKs, and HCN2 and HCN4/f-channel/If have been experimentally confirmed to be targets of miR-1 or miR-133. MiR-1 is intimately involved in the regulation of the cardiac conduction system and controls expression of the cell-to-cell communication protein connexin 43 being critical for inter-cell conductance of excitation, indicating that miR-1 is arrhythmogenic [37]. MiR-1 is overexpressed in patients with CAD, and when overexpressed in normal or infarcted rat hearts, it exacerbates arrhythmogenesis, at least partly through its post-transcriptional repression on KCNJ2 and GJA1, leading to slow conduction and unpolarized cytoplasmic membrane [95]. Overexpression of miR-1 by adenoviral-mediated in myocytes resulted in a marked increase in the amplitude of the inward Ca(2+) current, flattening of Ca(2+) transients voltage dependence, and enhanced frequency of spontaneous Ca(2+) sparks, while reduced the sarcoplasmic reticulum Ca(2+) content as compared with controls [96]. Down-regulation of miR-1 and miR-133 expression contributes to re-expression of HCN2/HCN4 regulating the pacemaker current I(f) of the hyperpolarization-activated channels, thereby contributing to the electrical remodelling process in hypertrophic hearts [97]. The high miR-133a levels increased QT intervals in surface electrocardiographic recordings and action potential durations in isolated ventricular myocytes, with a decrease in the fast component of the transient outward K(+) current, I(to,f), at baseline [98]. Administration of propranolol reversed the up-regulation of miR-1 nearly back to the normal level in the rat model of MI, resulting in the lessening of myocardial injuries, membrane depolarization recovery and cardiac conduction slowing during ischaemia, by rescuing the expression of inward rectifying K(+) channel subunit Kir2.1 and gap junction channel connexin 43 [99]. This finding implies that the  $\beta$ -adrenoceptor-cAMP-protein kinase A signalling pathway can stimulate expression of arrhythmogenic miR-1, while  $\beta$ -blockers produce their beneficial effects partially by down-regulating arrhythmogenic miR-1. Down-regulation of miR-1 with consequent recovery of Kir2.1 may also partially account for the efficacy of tanshinone IIA in suppressing ischaemic arrhythmias and cardiac mortality 3 months after MI in the rat model [100].

These findings shed new light on the cellular function and pathological role of miRNAs as potential therapeutic targets for the prevention of ischaemic arrhythmias, especially following MI. However, both MSCs and miRNAs as modulators of cardiac arrhythmias remain, at best, in its infancy. Consequently, further understanding the roles of miRNAs in the intrinsic electrophysiological properties of MSCs and both their integration with cardiac

tissue will one day make MSC-based therapeutics more effectively antiarrhythmic than presently available antiarrhythmic drug therapy.

## Preconditioning MSCs with MiRNAs as therapeutic perspectives

Although MSC-based therapy may be safe and hold promise in the future treatment of MI with resultant CHF, current evidence has shown that the efficacy of MSC transplantation is modest and it may only be considered as an adjunctive therapy at present. The poor viability and the rapid and massive loss of donor MSCs after engraftment due to harsh microenvironments such as shortage of oxygen and nutrients, oxidative stress, inflammatory response, and others, in the infarcted myocardium, may be the major obstacles in the clinical application of MSC-based therapy. It is therefore imperative to develop strategies to promote cell survival by programming and priming the cells before transplantation to withstand these rigors especially during the acute phase after engraftment to allow an interval long enough for the cells to acclimatize and engraft. The approach of cellular preconditioning has powerful cytoprotective effects and the signalling pathways involved in cellular preconditioning can be successfully employed for prosurvival measures in MSC-based therapy in MI. New technologies that utilize artificial miRNAs target sites to exploit or inhibit endogenous miRNA regulation of MSCs may also provide hope to alter this inefficient process. Preconditioning of MSCs with diazoxide significantly improved cell survival *via* NF-kappaB signalling, while blockade of miR-146a expression by antisense miR-146a inhibitor abolished diazoxide-induced cytoprotective effects, suggesting a critical role of miR-146a in MSC survival [101]. MiR-146a targeting Fas mRNA to down-regulate Fas protein expression may, at least partly, account for this efficacy. Another study has revealed that ischaemic preconditioning (IPC) significantly reduced apoptosis in MSCs through activation of Akt and ERK1/2 and nuclear translocation of HIF-1 $\alpha$ , concomitant with induction of miR-210 [102]. The authors also found that preconditioned MSCs predominantly improved survival after engraftment in a rat model of acute MI with a role for miR-210 targeting FLICE-associated huge protein (FLASH)/caspase-8-associated protein-2 (Casp8ap2) genes. The study also revealed that induction of FLASH/Casp8ap2 in miR-210 knocked-down preconditioned MSCs resulted in increased cell apoptosis, showing that miR-210-induced FLASH/Casp8ap2 suppression is a significant regulator of cytoprotection afforded by IPC. Additionally, Nie *et al.* [103] have recently revealed that over-expression of miR-21, miR-23a and miR-210 could promote the survival of MSCs exposed to hypoxia and serum deprivation, whereas down-regulation of miR-21, miR-23a and miR-503 aggravated apoptosis of MSCs.

The discovery of miRNAs and their roles as important transcriptional regulators in CVD may provide a new means of manipulating MSCs fate and benefits post-transplantation into the infarcted heart. In addition, preconditioning MSCs by miRNA

modulation may enhance cell capacity to differentiate into CMCs and vascular cells, release more paracrine factors accompanied by activation of their receptors and related signalling molecules, leading to better cardiac repair after MSC therapy. Till now, several methods may be used to precondition MSCs before engraftment into the host infarcted heart, which has been reviewed in detail by other authors [3, 104]. However, further direct studies are prerequisite to having a thorough understanding of the role of miRNAs in the cell death mechanisms and thus improve the efficacy of miRNAs in MSC preconditioning for MI treatment.

## Future directions and concluding remarks

Over the past decade, both miRNA- and MSC-based therapeutic research have been the two most attractive fields in human disease, including MI. Accumulating evidence from animal experiments and clinical trials has shown that cell differentiation, paracrine effects and other potential effects, such as cardiac nerve sprouting, anti-arrhythmia and cell fusion, may account for the beneficial effects of MSC-based therapy for MI in a combinative manner rather than its components. However, treatment of MI with MSCs has acquired only marginal positive benefits in clinical trials, perhaps because MSCs have limited plasticity and their poor viability post-transplantation due to harsh microenvironments in the infarcted heart. Gain- and loss-of-function studies in animal models of MI have revealed profound and unexpected functions for miRNAs in numerous facets of cardiac pathological processes, including the regulation of stem cell differentiation, cardiac regeneration, neovascularization, apoptosis, cardiac remodelling, contractility and myocardial arrhythmias, and others. Therefore, in order to maximize the efficacy of MSC therapy after MI, extraordinary scientific and medical benefits are yielded by the successful combination of MSCs and miRNAs.

Given the obvious involvement of miRNAs in almost every facet of putative repair mechanisms in MSC-based therapy in MI, molecules specifically regulating cardiovascular miRNAs, either mimicking or antagonizing miRNA actions, will hopefully induce cardiovascular cell differentiation, enhance paracrine factor release and improve other potential benefits. Furthermore, the highly expressed miRNAs in microvesicles released from engrafted MSCs may directly account for MSC-mediated cardiac repair in a paracrine/autocrine manner [18]. Also, MSCs are attractive as a cellular vehicle for gene delivery, including traceable synthetic miRNAs. However, the miRNA expression profile is tightly controlled in a cell-specific, tissue-specific and developmental stage-specific manner, and the pattern of miRNA expression in MSCs is substantially different from other cell types, thus the findings of miRNAs in other cell types may not be completely applicable to MSCs. Just as van Rooij *et al.* [104] have reviewed that miRNAs have numerous molecular targets and the functionality of a miRNA is determined by the combined regulation of many

different genes, which raises the possibility that the targeting of a miRNA may perturb multiple cellular functions, some pathological and other beneficial effects. In addition, due to different miRNA microarrays for analyses, different animal models, different time points of the same models, accompanied by their disease status dependent expression, current findings of the same cell type derived from the same source of the same model, still present some controversies. The better understanding of the general principles, such as the differential regulation principle, co-regulation principle, coordinate principle and competitive principle, coupled with false positive miRNAs, will further widen the study of miRNA function in MI and other ischaemic heart diseases [51]. Despite that several hundred of miRNAs have been found to express in MSCs and the roles of some of these miRNAs in MSCs have also been revealed [12,13,18,35,103,105], the specific expression and functionality of the majority of miRNAs in MSCs has not been well elucidated, especially in MSCs post-transplantation in infarcted myocardium. Albeit little evidence has recommended the pros or cons of the modification of MSCs with miRNAs prior to transplantation into the host infarcted heart, the combination of MSCs and miRNAs may be a feasible and promising therapeutic strategy for cardiac repair after MI, but it is just in its sprouting state. The majority of cardiac-specific and cell-specific microRNAs have been reviewed by other authors in different experimental ischaemic heart diseases, including MI with subsequent heart failure, or in different cell models or cells types [3, 12, 17, 104–106]. However, few microRNAs have been reviewed in MSCs, especially in MI models treatment with MSCs, in these studies, with no exception to this study. Therefore, additional attention should be

paid to miRNA expression in MSCs and its relationship to the characteristics of MSCs, which will augment the opportunities to safely pursue them as therapeutic modalities during MSC-based therapy in MI. We have given only a brief outline of the feasibility that miRNAs may be used as novel regulators in MSC-based therapy for MI. Many of the above problems have not been extensively tested yet in *in vivo* MI models, let alone in clinical practice. Optimal answers to all of these problems may pave the way for future research in the field of regenerative cardiology. Diagnostic use and therapeutic modulation of individual miRNAs of MSCs in CVDs, especially in MI will hopefully become a reality in coming future,

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## Conflict of interest

There are no conflicts of interest.

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