Chapter

Mesenchymal Stem Cell-Derived Exosomes for Myocardial Infarction Treatment

Huifeng Zheng, Yimei Hong, Bei Hu, Xin Li and Yuelin Zhang

Abstract

Myocardial infarction (MI) is a major cause of morbidity and mortality in modern society. Over the past decades, mesenchymal stem cell (MSCs)-based therapy has shown promising results in the treatment of MI due to their unique properties of multi-differentiation ability, immune-privileged phenotype and paracrine activity. Recently, MSC-derived exosomes (MSC-EXO) have been proposed as a promising therapeutic strategy for MI with their ability to inhibit cardiomyocyte apoptosis and stimulate vascular angiogenesis. They also aid immunoregulation and rejuvenation of cardiomyocyte senescence by transporting their unique content such as proteins, lipids, and miRNAs. Compared with MSC transplantation, MSC-EXO administration has shown several advantages, including lower toxicity and immunogenicity and no risk of tumor formation. Nonetheless the potential mechanisms underlying MSC-EXO-based therapy for MI are not fully understood. In addition, lack of modification of MSC-EXOs can impact therapeutic efficacy. It is vital to optimize MSC-EXO and enhance their therapeutic efficacy for MI. We summarize the recent advances regarding biological characteristics, therapeutic potential and mechanisms, and optimal approaches to the use of MSC-EXOs in the treatment of MI.

Keywords: mesenchymal stem cells, exosome, myocardial infarction, treatment, therapeutic effect

1. Introduction

Myocardial infarction (MI) results in irreversible loss of cardiomyocytes due to a restricted blood supply and is the major cause of morbidity and mortality worldwide. It has been estimated to account for 80% of deaths in patients with ischemic heart disease worldwide, and its prevalence continues to increase every year leading to a significant medical, social, and financial burden [1]. Despite the availability of advanced surgical interventions and medications including primary percutaneous coronary intervention, angiotensin-converting enzyme drugs and β-blockers, there remains no effective means to prevent cardiomyocyte loss due to myocardial ischemia [2]. The only cure for this devastating disease is heart transplantation but this is restricted by its high cost, a shortage of donor hearts, and the occurrence of immune

rejection following transplantation [3]. Exploration of novel therapies for left ventricle remodeling and dysfunction following infarction is urgently needed.

Over the past decades, stem cell-based therapy has become a promising strategy to treat MI with significant progress made in animal studies and clinical trials [4–6]. Among all types of stem cell under investigation, mesenchymal stem cells (MSCs) have garnered huge interest due to their easy isolation, high reproductive activity, differentiation capability and immunomodulatory properties [7, 8]. MSCs can be isolated from multiple tissues or cells including bone marrow, adipose tissue, umbilical cord blood and even pluripotent stem cells [9–12]. There is accumulating evidence that MSCs are promising candidates for MI treatment [13–15]. More importantly, it is now widely accepted that the cardioprotective effects of MSC-based therapy in MI are due to their strong paracrine effects, rather than trans-differentiation ability [7, 16–18]. Therefore, researchers are increasingly huge interested in the therapeutic efficacy of MSC-derived bioactive molecules, especially exosome (EXO), that are considered major components of the paracrine effect in MSC-based therapy [19, 20]. EXO, a subgroup of extracellular vesicles (EVs), are 40–160 nm diameter membrane-bound vesicles that can be found in almost all biological fluids. It has been well documented that MSC-EXO exert their cardioprotective effects in MI by delivering diverse biological molecules, including non-coding RNA, DNA, lipids and proteins [21–24]. More importantly, compared with MSC transplantation, MSC-EXO have several advantages such as easier storage and transplantation, less immune rejection, minimum risk of immunogenicity and no risk of tumor formation [25]. We discuss the current understanding of the biological characteristics, therapeutic effects and potential mechanisms of MSC-based therapy in MI. We also highlight the current challenges and potential approaches to improve the efficacy and production of MSC-EXO in regenerative medicine to guide their future clinical application.

2. Characterization and Isolation of MSC-EXO

EVs are bilayer lipid membrane-bound subcellular vesicles released by all types of cells and present in all body fluids. According to MISEV2018, EVs are divided into “small EVs” (sEVs, <100 nm or <200 nm) and “medium/large EVs”(m/lEVs, >200 nm) respectively [26]. EXO are sEVs approximately 40–160 nm in diameter (100 nm on average) and the main subclass of EVs [27]. The biogenesis of EXO begins with inward budding to form an early endosome. Finally, EXO are built when multi vesicular bodies (MVBs, late endosomes) fuse with plasma membrane and are secreted into the extracellular space [28, 29]. MSC-EXO express EXO-specific markers CD9, CD63, CD81, Alix and Tsg101 as well as MSC surface markers including CD29, CD44, CD90 and CD73. Among these, CD29 and CD44 have been identified previously as the specific biomarkers for MSC-EXO [30, 31]. The size and concentration of EXO can be characterized by nanoparticle tracking analysis (NTA) and transmission electron microscopy (TEM) [32]. Recently, plasmonic scattering microscopy has been applied to image exosomes and analyze biomarkers [33].

It is difficult to show whole landscape of EXO dispersed in solution. Therefore, purification of EXO is of importance for EXO definition. EXO are distributed throughout body fluids and this represents a challenge to their isolation. EXO are secreted into body fluids such as blood, urine, saliva, lymph, breast milk, cerebrospinal fluid and pericardial fluid etc. [34]. EXO components reflects the state of the original cell. Different methods of isolation of EXO varies from various body fluids. Meanwhile,

the extraction result differs from types of biological fluid. Which was optimal remains controversial [35]. Isolation of abundant EXO can help in the assessment of their biological functions [36]. Several recent alternative methods ranging from conventional to newly developed techniques to isolate and purify EXO are summarized in **Table 1**. Different methods for EXO isolation have different advantages and disadvantages. During isolation, ultracentrifugation and density gradient centrifugation are the most commonly used techniques [47]. Currently, several new methods have been established to facilitate high-throughput and high-purity manufacture of EXO. The characterization and isolation of MSC-EXO are summarized in **Figure 1**.

No.	Methods of EXO isolation	Advantages and disadvantages	Ref.
1	Ultracentrifugation	1. Most widely used 2. Gold standard for exosome separation 3. A series of speed centrifugation 4. Time consuming	[37]
2	Density gradient centrifugation	1. Sorts: Sucrose density gradient Iodixanol density gradient Optiprep density gradient 2. Improve purity of exosomes 3. Sucrose density gradient cannot effectively separate EXO and retroviruses 4. Time consuming and complex procedure	[38]
3	Chromatography (size-based isolation techniques)	1. Sorts: Mini-size exclusion chromatography (mini-SEC) Size exclusion chromatography 2. Quick, easy, small material consumption 3. May be applied with other particles of similar size	[39]
4	Ultrafiltration (size-based isolation techniques)	1. Uses ultrafiltration membranes with different molecular weight cutoffs (MWCO) 2. Low cost and high enrichment efficiency 3. Low purity and non-specific binding of EXO	[40]
5	Tangential flow filtration (size-based isolation techniques)	1. Using a cutoff TFF cartridge 2. Fluid flows tangentially across the surface, avoiding filter cake formation 3. Fast and efficient 4. Volume is limited by the instrument dead volume	[41]
6	Polymer-based precipitation separation	1. Uses polyethylene glycol (PEG) as a medium 2. Easy to operate with short analysis time 3. Polymer is difficult to remove	[42]
7	Immunoaffinity chromatography (IAC)	1. Based on the specific binding of antibodies and ligands 2. Storage conditions of EXO are relatively harsh and are not suitable for large-scale separation of EXO	[43]

No.	Methods of EXO isolation	Advantages and disadvantages	Ref.
8	Microfluidic technologies	1. Sorts Physical-property-based microfluidics Immunoaffinity-based microfluidics 2. Miniaturization, integration, high-throughput capacity, low-time consumption 3. Specialized equipment needed	[44]
9	Deterministic lateral displacement separation	1. Uses tilted pillar arrays that generate a fluid bifurcation and a unique number of streamlines between the gaps 2. Low separation throughput; particle adhesion and clogging; complex and bulky experimental setup	[45]
10	Acoustic fluid separation	1. Uses ultrasound waves to exert radiation forces on particles 2. Highly controllable, and versatile 3. The device is relatively low in a single channel microfluidic device	[46]

Table 1.
Methods of MSC-EXO isolation.

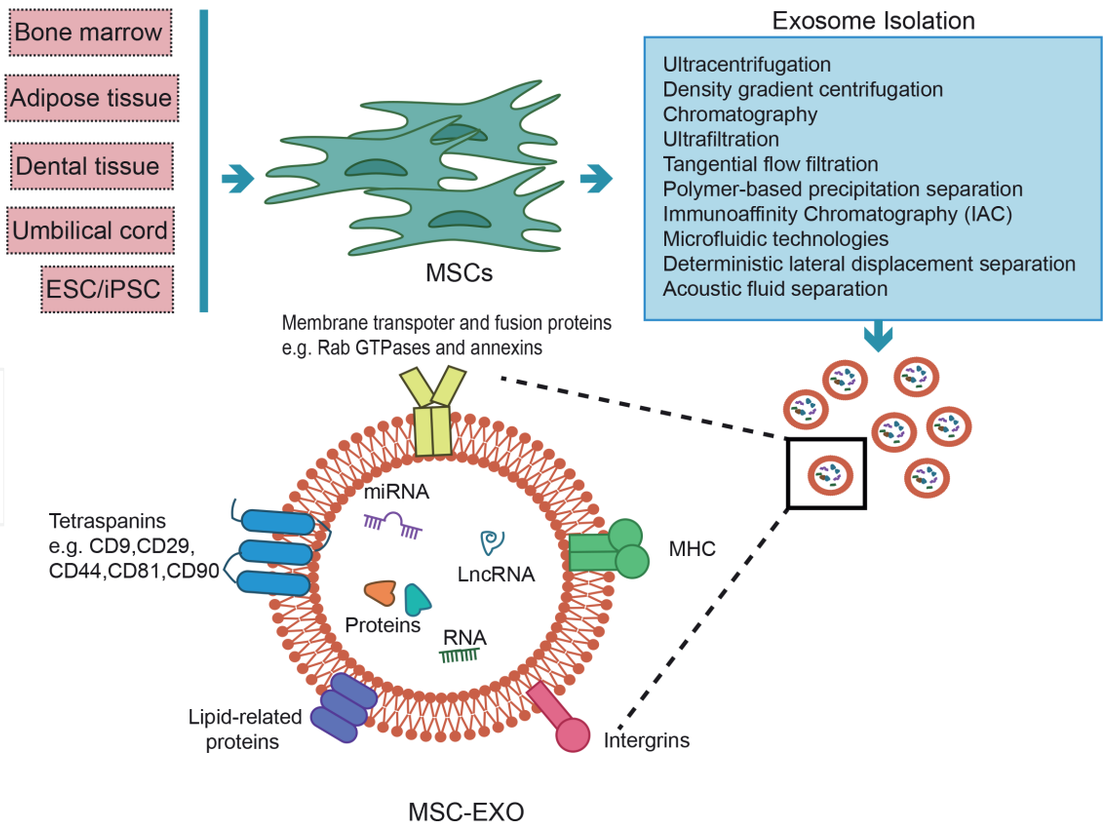

Figure 1.
Characterization and isolation of MSC-EXO.

3. The bioactive constituents of MSC-EXO for MI treatment

MSC-EXO exert their benefits in various diseases by enclosing and transporting a vast array of molecules [48]. It has been demonstrated that exosomal components are almost dependent on the source cell and cellular conditions [25, 49, 50]. Generally, EXO contain multiple characteristic molecules with typical physiological functions [51–53]. MSC-EXO comprise a variety of substances, including many kinds of proteins and a lot of noncoding RNA, including microRNAs (miRNAs) and long noncoding RNAs (lncRNA) [54]. These components can act as paracrine factors, mediating cell-to-cell signaling and communication. More importantly, they can be used as prognostic and diagnostic markers [55, 56].

3.1 Exosomal miRNAs in MSC-EXO for MI treatment

miRNAs are endogenous and 19–25 nucleotides in size. They can be isolated from cells, tissues and body fluids [57]. By pairing to the mRNAs of protein-coding genes, miRNAs play an important role in regulating post-transcriptional silencing of target genes [58, 59]. There is accumulating evidence that miRNAs are enriched in MSC-EXO and are the major bioactive constituents [60–62]. In the last few decades, the cardioprotective role of MSC-derived exosomal miRNAs has attracted huge attention [63]. It has been well documented that many MSC-derived exosomal miRNAs have beneficial functions in MI treatment [64]. Importantly, several potential mechanisms have been identified such as promotion of angiogenesis, reduction of cell death and an antifibrotic effect [65]. Enhanced angiogenesis is one of the important repair mechanisms underlying MSC-EXO-based therapy for MI [66–68]. Through direct miRNAs transfer, MSC-EXO convey their proangiogenic signals to injured cardiomyocytes [69]. Previous study has shown that silenced MSC-derived exosomal miR-210 largely lost its proangiogenic effect. Further experimental study revealed that exosomal miR-210 improves angiogenesis of MSC-EXO via targeting of Efna3 [70]. Zhu et al. demonstrated that macrophage migration inhibitory factor (MIF) could enhance the pro-angiogenic effect of MSC-EXO by enhancing the level of miR-133a-3p via regulation of the AKT signaling pathway [71]. miR-221 is one of the most studied miRNAs. A recent study reported that up-regulated exosomal miR-221-3p derived from senescent MSCs improved their ability of angiogenesis, migration and proliferation, and suppressed apoptosis by regulating the PTEN/AKT pathway [72]. Ma et al. revealed that miR-132-electroporated MSC-EXO could promote angiogenesis both *in vitro* and *in vivo* by downregulating RASA1 [23]. These studies show that MSC-EXO improve angiogenesis by transmitting miRNAs via various biological signaling pathway following MI.

There is increasing evidence that ameliorating cardiomyocyte death is another major mechanism by which EXO restore cardiac function following MI. MSC-EXO reduce myocardial cell death via multiple mechanisms including an anti-apoptosis action, inhibition of pyroptosis and an anti-inflammatory effect [73]. Apoptosis is programmed cell death that is strongly associated with myocardial ischemia [74]. Previous studies have proved that MSCs have an anti-apoptotic effect through secretion of exosomes enriched in miRNAs [75]. Hypoxia-elicited MSC-EXO (Hypo-EXO) facilitates cardiac repair by preventing cell death in MI via delivery of miR-125b.

Mechanistically, miR-125b-5p suppresses apoptosis of cardiomyocytes by down-regulating the expression of apoptotic genes p53 and BAK1 [63]. Another study demonstrated that EXO derived from miR-146a-modified adipose-MSCs attenuated MI via inhibition of apoptosis, the inflammatory response, and fibrosis in a rat model of AMI by targeting early growth response factor 1(EGR1) [76]. Wang et al. reported that adipose-MSC-EXO carrying miR-671 reduced the apoptosis of cardiomyocytes and alleviated myocardial fibrosis and inflammation via inactivation of the TGFBR2/Smad2 Axis [77]. miR-153-3p plays an important role in modulating cell proliferation, apoptosis and angiogenesis. It has been illustrated that EXO-miR-153-3p significantly reduces apoptosis of endothelial cells and cardiomyocytes and promotes their viability. By targeting ANGPT1, miR-153-3p can regulate the VEGF/VEGFR2/PI3K/AKT/eNOS pathways to prevent hypoxic damage to endothelial cells and cardiomyocytes [78]. Furthermore, a growing number of studies have shown that stem cell-derived exosomal miRNAs, such as miR-150-5p, miR-126, and miR-486-5p, demonstrate antiapoptotic activity in MI treatment [79–81]. These findings indicate that the anti-apoptotic effect of MSC-EXO can be partly ascribed to the delivery of some anti-apoptotic miRNAs.

Autophagy is a self-destructive process during which a cell degrades and recycles unnecessary or dysfunctional cellular components [82]. Autophagy is involved in promoting cell death and exacerbates myocardial dysfunction following severe ischemic stress. There is accumulating evidence that MSC-EXO reduce cell death by mediating autophagy. Xiao et al. determined that MSC-EXO reduced autophagic flux in infarcted hearts via exosomal transfer of miR-125b by interfering with p53/Bnip3 signaling and protected cardiomyocytes against damage [83]. Liu et al. showed that miR-93-5p-enhanced ADSC-EXO had a greater cardioprotective effect by suppressing hypoxia-induced autophagy and inflammatory cytokine expression via targeting of Atg7 and Toll-like receptor 4 (TLR4), respectively [84]. Furthermore, Li et al. reported that exosomal miR-301 derived from MSCs protected against MI by inhibiting myocardial autophagy [85]. In addition, MSC-exosomal miRNAs exerted a cardioprotective effect in MI by attenuating cardiac fibrosis. Inflammation and subsequent fibrosis are important pathological reactions that result in scar formation post-MI. Human umbilical cord MSCs-EXO containing miR-29b have been shown to prevent cardiac fibrosis following MI, leading to a reduction in infarct size and improved cardiac function in a mouse model of MI [86]. Moreover, miR-671 carried by adipose-derived MSC-EXO has been proven to also reduce myocardial fibrosis and inflammation both *in vitro* and i*n vivo* [77]. The roles of MSC-exosomal miRNA and the potential mechanism for MI treatment are summarized in **Table 2**.

3.2 Exosomal lncRNAs in MSC-EXO for MI treatment

LncRNAs are defined as RNA transcripts >200 nucleotides without protein-coding potential. lncRNAs play important roles in regulating a variety of biological processes. Recent studies have shown that they participate in the initiation and progression of MI through regulation of gene expression at the epigenetic, transcriptional and post-transcriptional levels [87]. Moreover, MSC-derived exosomal lncRNAs have been shown to have cardioprotective effects for MI. LncRNA KLF3-AS1 in human MSC-EXO ameliorated pyroptosis of cardiomyocytes in a rat model of MI via regulation of the miR-138-5p/Sirt1 axis [88]. A recent study has illustrated that hypoxia promoted MSCs to secret lncRNA-UCA1-enriched EXO

Model	Sources of EXO	Related-effectors	Biological effects	Involved pathway	Ref.
MI mouse with LAD ligation	BM-MSCs	miR-210	Angiogenesis	Efna3	[65]
MI rat with LAD ligation	UC-MSCs	miR-133-3p	Angiogenesis Anti-apoptosis Anti-fibrosis	P-AKT	[66]
MI rat with LAD ligation	BM-MSCs	miR-221-3p	Angiogenesis Anti-apoptosis	PTEN/AKT pathway	[67]
MI mouse with LAD ligation	BM-MSCs	miR-132	Angiogenesis increase tube formation enhance neovascularization	RASA1	[18]
MI mouse with LAD ligation	BM-MSCs	miR-125b	Anti-apoptosis	P53 and BAK1	[58]
MI rat with LAD ligation	AD-MSCs	miR-146	Anti-apoptosis Anti-inflammation Anti-fibrosis	EGR1/TLR4/NFκB	[71]
MI rat with LAD ligation	AD-MSCs	miR-671	Anti-fibrosis Anti-inflammation	TGFBR2/Smad2	[72]
Vitro model	BM-MSCs	miR-153-3p	Anti-apoptosis Angiogenesis	ANGPT1-VEGF/PI3k/AKT/eNOS	[73]
MI mouse with LAD ligation	BM-MSCs	miR-150-5p	Anti-apoptosis	Bax	[74]
MI rat with LAD ligation	AD-MSCs	miR-126	Anti-apoptosis Anti-inflammation Anti-fibrosis Angiogenesis	_	[75]
MI rat with LAD ligation	BM-MSCs	miR-486-5p	Anti-apoptosis	PTEN/PI3K/AKT	[76]
MI mouse with LAD ligation	BM-MSCs	miR-125b	Decreasing autophagic flux	p53/Bnip3	[78]
MI rat with LAD ligation	BM-MSCs	miR-301	Inhibiting myocardial autophagy	—	[80]
MI mouse with LAD ligation	UC-MSCs	miR-29b	Anti-fibrosis	—	[81]

Table 2.
MSC-exosomal miRNAs for MI treatment.

that had a cardioprotective effect via the lncRNA-UCA1/miR-873-5p/XIAP axis. Furthermore, exosomal lncRNA-UCA1 in human plasma may be considered a potential noninvasive biomarker for the diagnosis of AMI [89]. Similarly, Huang et al. showed that Atorvastatin pretreatment enhanced the therapeutic efficacy of MSC-EXO in a rat MI model via up-regulation of LncRNA H19 by promoting endothelial cell function [90]. The roles of MSC-exosomal LncRNA and their potential mechanism in MI treatment are summarized in **Table 3**.

Model	Sources of EXO	Related-effectors	Biological effects	Involved pathway	Ref.
MI rats with LAD ligation	hMSCs	LncRNA KLF3-AS1	Amelioration of pyroptosis	miR-138-5p/Sirt1	[83]
MI rats with LAD ligation	hMSCs	LncRNA-UCA1	Anti-apoptosis	miR-873-5p/ XIAP	[84]
MI rats with LAD ligation	BM-MSCs	LncRNA H19	Anti-apoptosis Angiogenesis Anti-inflammation Anti-fibrosis	miR-675, VEGF and ICAM-1	[85]

Table 3.
MSC-exosomal LncRNAs for MI treatment.

3.3 Exosomal proteins in MSC-EXO for MI treatment

MSC-EXOs further elicit benefit by delivering their cargo of potentially therapeutic proteins to recipient cells [91]. To date, nearly two thousand proteins in MSC-EXO have been identified [92–96]. Like miRNAs and lncRNAs, proteins in MSC-EXO have the potential to protect cardiomyocytes against injury following MI. Proteins in MSC-EXO whose role is basic cellular function, include common proteins, enzymes and signaling molecules [97]. One study suggested that hucMSC-EXO protected myocardial cells against apoptosis and promoted cell proliferation and angiogenesis by improving the expression of Bcl-2 family [98]. EXO secreted from CXCR4 overexpressing MSCs have been shown to promote cardiomyocyte survival and angiogenesis in ischemic hearts following MI via the AKT signaling pathway [99]. Deng et al. reported that EXO from AD-MSCs could ameliorate cardiac damage following MI by activating S1P/SK1/S1PR1 signaling and promoting macrophage M2 polarization [100]. The roles of MSC-exosomal proteins and their potential mechanism for MI treatment are summarized in **Table 4**.

Taken together, although current knowledge is limited, it can be inferred that various proteins carried by MSC-EXO protect ischemic cardiomyocytes through different mechanisms.

Model	Sources of EXO	Related-effectors	Biological effects	Involved pathway	Ref.
MI rats with LAD ligation	UC-MSCs	Bcl-2 family, Ki67	Anti-fibrosis angiogenesis	—	[98]
MI rat with LAD ligation	BM-MSCs	DMBT1	Promotes angiogenesis	PI3K-AKT/ GSK3β/β-catenin/ VEGF	[99]
MI rat with LAD ligation	AD-MSCs	S1P, SK1, S1PR1	Anti-apoptosis anti-fibrosis anti-inflammation promotes macrophage M2 polarization	S1P/SK1/S1PR1	[100]

Table 4.
MSC-Exosomal proteins for MI treatment.

4. Potential strategies to improve the therapeutic efficacy of MSC-EXO for MI

Although MSC-EXO-based therapy has shown promising results in MI, their therapeutic efficacy is heavily restricted by the low production and concentration of biological molecules released by EXO derived from MSCs under routine culture conditions. The production and biological components of MSC-EXO vary depending on the different external stimuli surrounding MSCs and MSC status. Therefore, modifying and optimizing exosomal content in MSC-EXO *in vitro* prior to transplantation to enhance their therapeutic efficacy for MI is vital. Over the past decades, several novel strategies, including altering culture conditions and pretreatment with pharmacological compounds and molecules, have been explored to generate modified MSC-EXO with greater benefits for MI treatment [56, 101]. More importantly, genetic modification of MSCs has had a great impact on the release of MSC-EXO, directly modulating their therapeutic efficacy. The influence of these factors on production and function of MSC-EXO will be discussed in the following sections. Different strategies to improve the therapeutic effects of MSC-EXO in MI are summarized in **Figure 2**.

4.1 MSC-EXO generated from different culture conditions

The status of MSCs is largely dependent on culture conditions. Changes to culture conditions may influence MSC-EXO content and its biological functions. As a key impact on MSC culture, oxygen concentration plays a critical role in the regulation of gene expression, exon splicing, and phenotype of MSCs [102]. Therefore, oxygen gradients control MSC functions and generate different biological functions of MSC-EXO. MSCs survive under hypoxic conditions after transplantation into the ischemic heart and then release EXO to exert their benefit. Nonetheless MSCs are usually cultured under normoxic conditions *in vitro.* Therefore, the EXO released from MSCs under normoxic conditions *in vitro* and under hypoxic conditions *in vivo* carry different biological molecules with correspondingly different therapeutic effects. It has been reported that transplantation of MSCs under hypoxic conditions results in an enhanced therapeutic effect for MI [103, 104], indicating that hypoxic preconditioning may be a potential approach to prime MSC-EXO for MI treatment. Accumulating

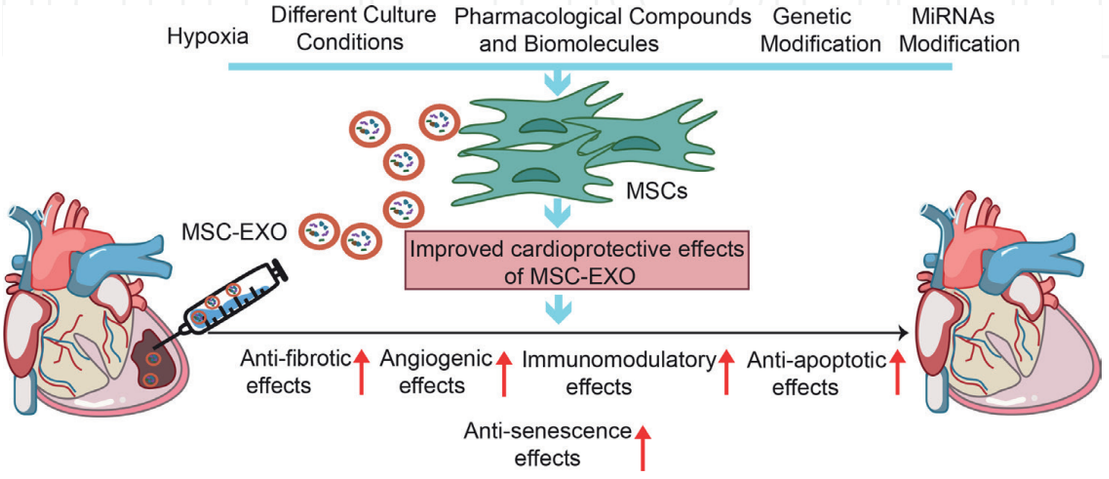

Figure 2.
Different strategies to improve the therapeutic effects of MSC-EXO in MI.

evidence shows that EXO from hypoxia-primed MSCs used to treat MI are superior to EXO from MSCs cultured under normoxic conditions [63, 105, 106]. Hypoxic preconditioning can enrich some specific miRNAs in the MSC-EXO that protect against MI by promoting angiogenic potential, attenuating inflammation and ameliorating apoptosis of cardiomyocytes [101]. It has been documented that hypoxic preconditioning of MSC-EXO elicits better therapeutic efficacy for MI by reducing the apoptosis of cardiomyocytes via upregulation of miR-210 that targets AIFM3 protein [75]. Zhang et al. showed that EXO isolated from hypoxic MSCs improved myocardial function in a rat model of myocardial ischemia-reperfusion injury by suppressing oxidative stress and the inflammatory response via delivery of miR-98-5p [107]. More importantly, EXO derived from MSCs stably overexpressing hypoxia inducible factor (HIF)-1α displayed an increased angiogenic capacity, partially due to the high level of Jagged1. This may have potential applications for MI treatment [108]. Indeed, transplantation of EXO collected from HIF-1α overexpressing MSCs improved heart function by promoting angiogenic formation in a rat model of MI [109]. Apart from hypoxic conditions, culture medium with different types of serum influence the characteristics of MSCs, modulating the efficacy of MSC-EXO-based therapies. Compared with normal serum, MSCs cultured with serum collected from the blood of mice with middle cerebral artery occlusion robustly demonstrated an upregulated level of miR-20a in their EXO [110]. Whether culturing MSCs with special serum can improve the efficacy of MSC-EXO for MI remains to be determined. Recently, it has been reported that the production of MSC-EXO can be augmented using a 3D porous scaffold structure instead of the traditional 2D culture in plastic plates, providing a novel strategy to optimize MSC-EXO for MI treatment [111]. Therefore, exploring suitable culture conditions for MSCs will not only improve the yield of EXO but also modify the therapeutic components of the EXO, ultimately enhancing their efficacy for MI treatment.

4.2 MSC-EXO generated following preconditioning with pharmacological compounds and biomolecules of MSCs

There is accumulating evidence that preconditioning with pharmacological agents and biomolecules robustly improves the therapeutic efficacy of MSCs in MI by enhancing MSC survival and paracrine effects [112–115]. These results prompted us to determine whether pharmacological preconditioning could be a novel approach to enhance the cardioprotective effects of MSC-EXO. Our group has shown that compared with MSC-EXO, EXO isolated from MSCs pretreated with hemin, a potent heme oxygenase-1 (HO-1) inducer, exhibited better cardioprotection for MI via inhibition of cardiomyocyte senescence by elevating the level of miR-183-5p [116]. Huang et al. demonstrated that EXO obtained from atorvastatin-pretreated MSCs had greatly enhanced therapeutic efficacy for MI treatment in terms of promoting angiogenesis and inhibiting inflammation [90]. In addition to pharmacological agents, preconditioning with specific biomolecules can contribute to the secretion of MSC-EXO. EXO derived from interferon-gamma (IFN-γ)-treated MSCs exhibited more potent cardioprotective function in a rat model of MI by increasing angiogenesis and inhibiting cardiomyocyte apoptosis through upregulation of miR-21 [117]. Interestingly, Xiao et al. found that compared with MSC-EXO, EXO derived from MSCs pretreated with ischemic rat heart extracts enriched with IL-22 promoted the angiogenic capacity of human umbilical vein endothelial cells, indicating a novel preconditioning approach to optimize MSC-EXO for MI treatment [99]. These reports

confirm that preconditioning with pharmacological compounds or biomolecules can alter the surrounding microenvironment of the culture conditions of MSCs and influence their paracrine effects, ultimately affecting the action of their derived EXO.

4.3 MSC-EXO isolated from genetically modified MSCs

Genetic modification of MSCs via knockdown or overexpression of some RNAs or proteins is another efficient approach to improve the therapeutic effect of MSC-EXO. Our previous study showed that compared with MSC-EXO, administration of EXO secreted by MSCs transduced with macrophage migration inhibitory factor, a proinflammatory cytokine, exhibited a better therapeutic efficacy for MI by downregulating cardiomyocyte mitochondrial fragmentation, reactive oxygen species generation, and apoptosis [118]. A recent report revealed that EXO collected from stromal-derived factor 1-overexpressing MSCs intravenously administered in a mouse model displayed enhanced heart protection by inhibiting apoptosis and autophagy of myocardial cells and increasing angiogenesis by the regulating PI3K signaling pathway [119]. In another study, EXO from MSCs transduced with lentiviral CXCR4 promoted restoration of cardiac function in a rat model of MI by ameliorating cardiomyocyte apoptosis and increasing angiogenesis via upregulation of IGF-1α and p-AKT levels and downregulation of active caspase 3 level [120]. As discussed above, miRNAs are important biological components that play a pivotal role in the cardioprotective effect of MSC-EXO in MI [121–123]. Therefore, overexpression of miRNAs in MSCs can enhance the efficacy of MSC-EXO for MI treatment. Direct injection of MSC-EXO with miR-183-5p overexpression has been shown to result in better cardiac function via suppression of apoptosis and oxidative stress of cardiomyocytes by targeting FOXO1 [124]. Administration of EXO derived from miR-129-5p-modified MSCs displayed enhanced cardiac function following MI in mice by downregulating apoptosis of cardiomyocytes and production of inflammatory cytokines via targeting of HMGB1 [125]. Moreover, EXO derived from miR-126-overexpressing adipose-MSCs demonstrated better beneficial effects by inhibiting cardiac fibrosis and inflammatory cytokine expression and increasing angiogenesis [80]. Thus, genetically modified MSC-EXO have been considered an effective means by which to enhance their cardioprotective effects in MI.

5. Limitations and challenges of MSC-EXO-based therapy for MI

Despite several significant advantages over MSCs, there remain some limitations and challenges to the clinical application of MSC-EXO for MI treatment. First, the rapid clearance of MSC-EXO from ischemic heart tissue after transplantation limits the beneficial effects for MI. An optimum delivery route for administration of MSC-EXO is unavailable. Currently, intramyocardial transplantation is the most efficacious. Exploration of alternative approaches to optimize retention and engraftment of MSC-EXO in the ischemic heart is urgently needed. Second, although the biological components in MSC-EXO, including miRNAs, lncRNA, recombinant proteins, and cytokines, have been intensively investigated, the exact mechanisms underlying MSC-EXO-based therapy for MI require further investigation. Third, MSC-EXO are currently isolated mainly depending on their vesicle size. Different sizes of MSC-EXO may contain different components with corresponding different therapeutic outcomes for MI. A more accurate isolation and purification method for MSC-EXO

should be adopted. Fourth, multiple harmful and unwanted biological components in MSC-EXO may restrict their efficiency. Several strategies to modify and remove unwanted components are under investigation. Finally, although classic high-speed centrifugation is the most common method used for MSC-EXO isolation, it is limited by the disadvantages of low production of EXO, high heterogeneity and non-scalability. A scalable isolation protocol for mass production of homogenous MSC-EXO for clinical application is needed.

6. Conclusion

Over the past decades, administration of MSC-EXO has been shown to attenuate cardiac remodeling and improve heart function recovery following MI by inhibiting cardiomyocyte apoptosis, stimulating vascular angiogenesis, immunoregulation and rejuvenating cardiomyocyte senescence. Although the great potential of MSC-EXO therapy for heart function recovery has been clearly demonstrated, the therapeutic role of MSC-EXO in MI is extremely complex. Many issues remain to be carefully addressed and evaluated including the need for a high quality isolation protocol, delivery routes, and optimum EXO dose. In addition, potential risks must be carefully evaluated prior to translation into clinical trials. MSC-EXO-based therapy is still in its infancy and most experimental studies have been in a small animal model. The therapeutic efficacy of MSC-EXO should be evaluated in a porcine model or pre-clinical large animal model. This may provide further evidence to support clinical translation of MSC-EXO-based therapy to humans. Despite the unresolved issues, with the advanced development and technical breakthroughs in EXO research, it is hoped that clinical translation of MSC-EXO to promote cardiac regeneration and repair will soon be a reality for patients with MI.

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Conflict of interest

The authors confirm that they have no conflicts of interest.

Other declarations

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