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*Review*

# Mesenchymal stem cell-derived exosomes: A novel potential therapeutic avenue for cardiac regeneration

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**Abstract:** Coronary artery diseases (CADs) represent a significant cause of death worldwide. During recent decades the rate of cardiovascular mortality has been declined as a result of modern medicine and surgery. However, despite the fact that cardiac cells, including cardiomyocytes (CMCs), vascular smooth muscle cells (VSMC) and vascular endothelial cells (VEC), can be regenerated by cardiac adult stem cell, the regenerative capacity of these cells are limited and inadequate to functionally regenerate heart damaged tissue. Thus, growth reserve of the heart fails to restore the structural integrity of the myocardium after infarction and healing is associated with scar formation. An explanation for this is that cardiac reside stem cells are present throughout the infarction site but die rapidly by apoptosis. Furthermore, microenvironment surrounding the damage site is not promising for the cells survival and renewal. Hence, recent advances in the stem cell therapy have emerged as an attractive approach to replace the lost cells. In this context, mesenchymal stem cells (MSCs) has considered as one of the most promising candidates for regeneration of cardiac cells, lost upon injury. The regenerative capacity of MSCs has primarily been centered on the hypothesis that these cells would engraft, differentiate and replace damaged cardiac cells. However, experimental and clinical observations so far have failed to establish if this differentiated is considerably relevant to MSCs cardiac regenerative properties. Recent reports have suggested that these therapeutic properties, at least in part, are mediated by paracrine factors released from MSCs. This review provides a concise summary of current evidences supporting the paracrine hypothesis of MSCs. In particular, the scope of this review focuses on the role of MSC-derived exosome (MSC-EXs) as a therapeutic modality for the treatment of CADs, particularly ischemic myocardial dysfunctions.

***Key words:*** Coronary artery diseases, cardiac regeneration, mesenchymal stem cells, exosome.

## Introduction

Coronary artery diseases (CADs), also known as ischemic cardiac disease (ICD) account for >17 million deaths globally each year (30% of all deaths) (1). It is clinically manifest as acute myocardial infarction (AMI), stable angina, unstable angina and sudden cardiac death (SCD). The underlying pathology of CADs is formation of plaque within coronary artery - a condition called atherosclerosis - that leads to blockages of blood flow. Regional myocardial ischemia, subsequent to a significant reduction or cessation of coronary arterial blood flow, induces CMCs loss in the segment supplied by the coronary artery.

Unfortunately, myocardial infarction (MI) endogenous cardiac repair via differentiation of residing cardiac progenitor cells (CPCs) or renewal of pre-existing adult CMCs appears to be limited by insufficient number of these cells. Moreover, although many life-saving cardiovascular therapies such as pharmacotherapy and surgical treatments are widely available, they all fail to restore or regenerate damaged cardiac tissue. Thus, the fact remains that after a loss of myocardial cells, the resulting necrotic and scar tissue cannot be restored properly. Therefore, the ideal therapeutic approach would aim to replace the lost cells and restore the cardiac normal function.

Stem cell-based regenerative medicine represents a new paradigm approach in treatment of cardiac injuries. Currently, several types of stem cells, including cardiac-derived stem cells (CSCs), bone marrow-derived stem cells, MSCs, skeletal myoblasts (SMs), and hematopoietic stem cells (HSCs) have been applied in clinical researches (2-4).

MSCs have been shown to be a stem cell population with great promise for cardiac regenerative applications (5). Several preclinical and clinical studies have supported the hypothesis that cardiac transfer of MSCs can have a favorable impact on cardiac function after injury (6, 7). The initial focus of therapeutic effects of MSC was based on their ability to differentiate into multiple cardiovascular cell lineages including VEC, VSMC and CMCs (8-10). Basically, the expectation was that upon implanting or injecting MSC, the cells would colonize at the lesion site and differentiate to appropriate cardiovascular cells. However, recently this mechanism has been challenged as MSCs do not persist well inside ischemic microenvironment of injured tissues and if there is low or no incorporation into the host tissue, most of the cells are lost within a month (11). Furthermore, no direct *in*

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*vivo* evidence was obtained in support of differentiation of MSC to cardiovascular cells at the site of injury (12). In conclusion, MSCs differentiation into functional cardiovascular cells seems to be inefficient solely to exert therapeutic effects on extensive cardiovascular injuries resulted from ischemia.

In recent years it has been suggested that MSC produce components with biological activities that appear to mediate their therapeutic effects. This so called "paracrine hypothesis", has gained much attention and is greatly supported by experimental evidences (13-17). Even though, the initial attention on paracrine therapeutic potential of MSC had centered on factors such as chemokines, cytokines and growth factors, none could sufficiently explain the efficacy of MSCs against the complex pathological processes of cardiac injury. Thus, recent efforts to investigate the therapeutic properties of MSCs secretome have shifted from these molecules to more complex structures, known collectively as extracellular vesicles (EVs).

EVs consist of several classes of secreted vesicles such as exosomes, microvesicles, ectosomes, membrane particles, exosome-like vesicles and apoptotic bodies. Among EVs, exosomes are, by far, the best characterized for their potential utility as cell-free therapeutic candidates that can mediate cardiac regeneration.

In the present review we will provide a brief discussion about MSCs cell-based therapy for cardiac regeneration followed by a deeper analysis of MSCs paracrine effects, with central focus on exosomes in this regard.

## Administration of mesenchymal stem cells in cardiac regeneration

Despite recent studies, suggesting that the heart has intrinsic mechanisms of self-regeneration following loss of cardiovascular cells, unfortunately after a major tissue injury, it cannot regenerate itself to the optimal level. In clinical practice, the concept of regenerative medicine and tissue repair using stem cells has increased considerably in the last decade. In the context of cardiac regeneration, the ultimate goal is to restore perfusion, contractility, lusitropy and conduction by repopulation of injured tissue with normal and healthy cardiac cells. Across different types of stem cells, MSCs are indicated to be a promising stem cell candidate for repairing cardiac tissue damages (8, 18, 19). They can be easily isolated from bone marrow, adipose tissue, lung, liver, tendons, placenta, amniotic fluid, dental pulp, synovial membrane and heart (20, 21). The advantages of MSCs in clinical use are due to their easily isolation, multilineage differentiation potential, low immunogenicity, immunosuppression effects, low tumorigenicity and lack of ethical controversy.

Cardiac repair after MSC transplantation is well described in both preclinical models and clinical trials (22). Moreover, in a recent phase I/II randomized, double-blind, single-dose study, the intravenous administration of allogeneic MSCs in patients with MI was reported to be safe and well tolerated (6).

Available data suggest multiple beneficial effects of MSCs in cardiac regeneration, which together lead to the repair of scarred or dysfunctional cardiac tissue (23). Currently four mechanisms are suggested to be involved in MSCs cardiac repair properties.

### *Differentiation of MSCs to cardiovascular cells*

The ability of MSCs to differentiate into cardiovascular cells including CMCs, VSMC, and VEC (24, 25) is subjected to controversial results. In 2001, Toma et al (26) reported that only a limited number of human bone marrow derived MSCs (BM-MSCs), injected into the left ventricle of immunodeficient adult murine, were engrafted within the myocardium (0.44%) and differentiated into the CMCs. In other study, Quevedo et al. (27) reported that only a small number of allogeneic MSCs can engraft, survive and differentiate to into CMCs, VSMC, and VEC in a swine model of chronic ischemic cardiomyopathy. In contrast to available reports of rare engraftment and differentiation of MSCs, some other studies indicated no engraftment of the MSCs injected within the cardiac borderzone in animal model of cardiac injury (28). It is suggested that, due to ischemic microenvironment of injured tissues that poses a serious problem for MSCs survival, these cells do not persist for long inside the environment of damaged myocardium (11). In fact, the regenerative efficacy of MSC transplantation in repairing tissue damages has been increasingly observed to be dependent on other mechanisms rather than engraftment and differentiation.

### *Cell-Cell interactions and CSCs stimulation*

The ability of MSCs to stimulate proliferation of endogenous CSCs and restore the functional CMCs has been indicated in both animal model of MI and co-culture experiments with CSCs and MSCs (29). In this regard, cell-cell interactions are suggested to play an important role in CSCs stimulation by MSCs. However, other available studies have reported that stimulation of endogenous CSCs via MSCs is infrequent or absent in vivo (30), which is due to the fact that ischemic microenvironment is a major obstacle for MSCs physical interaction with cardiac resident cells (19, 30, 31).

### *MSC and CMCs fusion*

Fusion of MSCs with adult cells, including CMCs, has been proposed as a regenerative approach. However, available studies report that this phenomenon is a rare and inefficient process to cope with the major loss of CMCs after MI or other ICD, ruling out any significant involvement in MSCs cardiac regeneration effects (19, 30).

### *Paracrine signaling*

Following indication of transient engraftment, low levels of MSCs stimulation of CSCs and infrequent MSCs fusion with CMCs, other possible cardiac regeneration mechanism remains to be MSCs paracrine effects (13).

Currently paracrine effects are implicated as the most comprehensive and enduring mode of action for MSCs therapeutic modality in cardiovascular repair. MSCs are known to secrete a wide array of cytokines and growth factors, which can suppress the immune response, inhibit fibrosis and cell apoptosis, enhance angiogenesis, activate cell proliferation, and stimulate differentiation of tissue specific stem cells. This complex secretome of MSCs is released either directly or packaged in EVs

(32).

EV is a term recently proposed by Gyorgy et al. (33) to describe the membrane surrounded structures released by several cell types and classified by their size and composition. Current research on therapeutic use of EVs released from MSCs has focused principally on microvesicles (MVs) and exosomes, although other vesicular structures can be secreted, among them microparticles and apoptotic bodies. At present, MSC-EXs are forefront of research in the field cardiac regeneration and repair.

The potential cardiac regenerative benefits of MSCs secretome components was first reported by Gnecchi et al. (19, 34) who observed that the administration of BM-MSCs leads to cardiac functional improvement in less than 72 hours post MI. As the immediacy of this protective effect cannot be explained by meaningful cardiac regeneration, resulting from differentiation of transplanted MSCs, the investigators hypothesized that MSCs paracrine actions might be important mechanisms of tissue repair and functional improvement. In support of this paracrine hypothesis, following studies showed that MSCs secret multiple bioactive molecules, which could potentially repair injured cardiac tissue mainly through cardiac and vascular tissue regeneration (35, 36). However, because of the diversity and complexity of the paracrine factors, it was not easy to identify which paracrine factor(s) play critical roles in cardiac repair process (13).

In 2007, Timmers et al. (35) for the first time suggested that the active cardioprotective component in MSC culture medium is a complex of multiple factor(s) with size ranging from 100 to 220 nm rather than a single molecule. In their following study (37) the investigators demonstrated that phospholipid vesicles, with diameter of with diameter of nano-metric range , are the cardioprotective component in MSC paracrine secretion. These nano-size particles were called exosome.

## Exosomes

Exosomes are endosomal-origin small-membrane vesicles with a diameter of 40 to 100 nm and a density in sucrose of 1.13–1.19 g/ml, which can be sedimented at 100,000 g. Exosomes are secreted from many cell types and are present in many and perhaps biological fluids, including blood, urine, and cultured medium of cell cultures. They share an evolutionary conserved set of proteins including tetraspanins (CD81, CD63, and CD9), Alix and Tsg101 as well as coding/noncoding RNAs unique to their cell source and the pathophysiological states. Their membranes are enriched in cholesterol, sphingomyelin and ceramide, and are known to contain lipid rafts. As lipid vesicles, exosomes were suggested to represent an ideal vehicle for delivery of functional cargo molecules and play important roles in intercellular communications. Exosomes contain many different cell surface molecules and are able to engage many different cell receptors simultaneously. This allows them to participate in the exchange of materials between cells.

Exosome formation is a fine-tuned process which includes four stages: initiation, endocytosis, multivesicular bodies (MVBs) formation, and exosome secretion

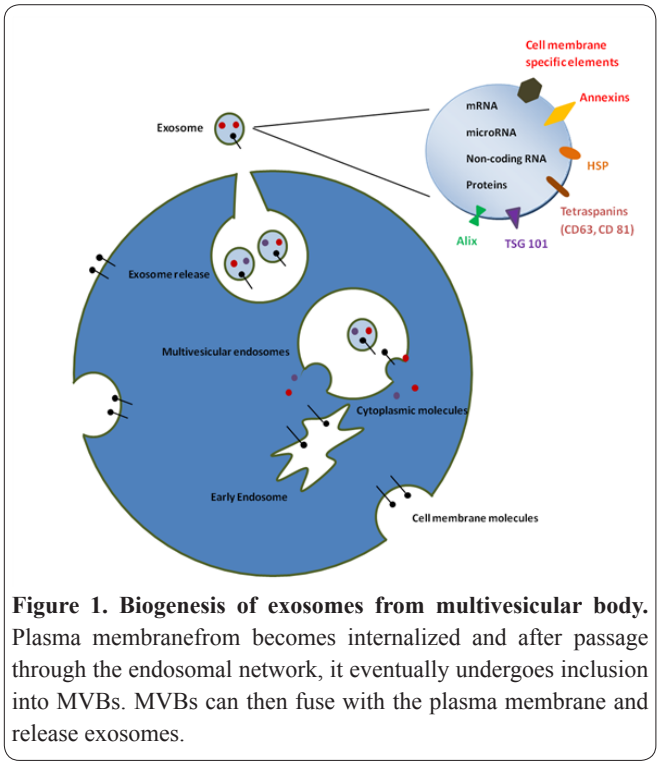


**Figure 1. Biogenesis of exosomes from multivesicular body.** Plasma membranefrom becomes internalized and after passage through the endosomal network, it eventually undergoes inclusion into MVBs. MVBs can then fuse with the plasma membrane and release exosomes.

(Figure 1). MVBs which appear along the endocytic pathway are formed by the inward budding of an endosomal membrane into the lumen of the compartment, thus, these vesicles are characterized by the presence of vesicles in their lumen. After vesicular accumulation, the MVBs are either sorted for cargo degradation in the lysosome or released into the extracellular space as exosomes by fusing with the plasma membrane. The mechanisms underlying cargo clustering into the intraluminal vesicles (ILVs) are not yet fully elucidated. Both endosomal sorting complex required for transport (ESCRT)-dependent and independent signals have been suggested to determine the sorting of Exosomes (38). Moreover, the syndecan heparan sulfate proteoglycans and their cytoplasmic adaptor syntenin have been indicated to control the formation of exosomes (38). The Rab guanosine triphosphatases (GTPases) have been found to critically regulate exosome secretion.

The function of the original cell from which an exosome originate can be deduced by the makeup of proteins, lipids, miRNAs, mRNA, non-coding RNAs and other molecules found in a particular exosome. Both ubiquitous and cell-specific membrane molecules (lipids and proteins) and loaded cargos (proteins, mRNAs, non-coding RNAs) are targeted selectively to exosomes. The most common proteins, mRNA, and miRNAs found in exosomes have been deposited in ExoCarta (www.exocarta.org). The current version of ExoCarta hosts 41,860 proteins, >7540 RNA and 1116 lipid molecules from more than 286 exosomal studies annotated with International Society for Extracellular Vesicles. The exosomal contents vary between different physiological and pathological conditions and original cell types.

At present, the most commonly used methods for exosome isolation include ultracentrifugation, combined with sucrose gradient, and the immune-bead isolation (e.g., magnetic activated cell sorting; MACS). There are many commercial kits available for the extraction of exosomes. Transmission electron microscopy (TEM),

**Table1.** Cardiac regeneration benefits of MSC-Ex cargo.

| MSC-Ex Cargo | Function |
|---|---|
| enzymes of ATP-producing phase of glycolysis ATP-generating phase of glycolysis | Inhibition of cardiac cells apoptosis via increasing ATP generation (54) |
| CD73, an adenosine-generating extracellular enzyme | Activation of adenosine receptors and induce Akt/GSK-3β survival kinase signaling pathway (55) |
| peroxiredoxins and glutathione S-transferases | Lowering oxidative stress (56) |
| miR-22 | Interaction Mecp2 (57) |
| miR-221 | Inhibition of cardiac cell apoptosis via targeting PUMA (59) |
| miR-19 | Inhibition of PTEN, an inhibitor of the AKT survival signaling pathway (59) |
| Unspecified factor(s) | Inhibition of inflammatory response (61) |
| Unspecified factor(s) | Prevention of adverse cardiac remodeling (55) |
| Unspecified factor(s) | Neovascularization (43) |

Western blot, and FACS are frequently used to characterize the isolated exosomes based on their biochemical properties (e.g., morphology, size, exosomal markers).

Identification of MSCs secretion of exosomes has led to a growing body of studies on the therapeutic applications of these particles in regenerative medicine. Currently, MSCs-EXs derived from different cell sourses have been used for regeneration of acute kidney injuries (39) , skin wounds (40), liver-injury (41), traumatic brain injury (42) and myocardial damages (43). Following the identification of the role of exosomes in cardiac repair, growing body of researches investigated the regenerative and protective mechanisms of these nanovesicles (Table 1 and Figure 2).

Administration of exosomes has different advantages over stem-cell transplantation. Occlusion in the distal microvasculature and migration of cells into areas of normal tissue are two common side effects of direct tissue transplantation or intravascular administration of MSCs (44-46). Additionally, differentiation potential of MSCs into multiple mesenchymal lineages (i.e., osteocytes, chondrocytes, and adipose tissue) raises long-term safety concerns as their transplantation may lead to cardiac ossification and/or calcification.

In contrast to MSCs, exosomes are non-viable and would not differentiate into inappropriate cell lineage or form tumors. Therefore, administration of them as a cell-free approach in cardiac regeneration could mitigate many of the safety concerns and limitations associated with the transplantation of viable MSCs.

Besides, the efficiency of exosome uptake has been correlated to intracellular and microenvironmental acidity (47). This provides a mechanism for exosome homing to ischemic tissues whereby MSC exosomes are preferentially endocytosed by ischemic CMCs which have low intracellular pH (48).

Interestingly, exosomes bear tetraspanins CD9 and CD81on their membrane, which respectively direct them to CMCs that express intercellular adhesion molecule 1 (ICAM-1), a ligand of integrins after myocardial injury (49), and to vascular cell adhesion molecule-1 (VCAM-1) on endothelial cells (50). Besides, Tetraspanin proteins, which function primarily to mediate cellular penetration, invasion and fusion events, could facilitate cellular uptake of exosomes by specific cell types.

The use of viable replicating cells as therapeutic agents carries the risk that the biological potency of the

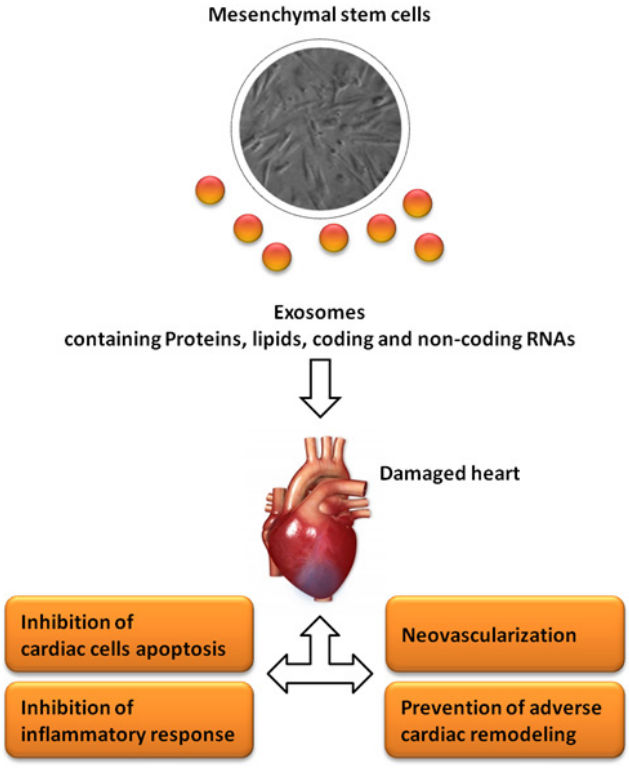


**Figure 2. Therapeutic advantageous of MSCs secretion (exosomes) in cardiac regeneration.** Exosomes derived from MSCs can suppress the immune response, inhibit apoptosis, enhance angiogenesis, activate cell proliferation, and prevent adverse cardiac remodeling.

agent may persist or be amplified over time when the need has been resolved, and cannot be attenuated after treatment is terminated (dose second draft)

Furthermore, despite being smaller than a cell, exosomes are relatively complex biological entities that contain a range of biological molecules, including proteins and RNA, making them an ideal therapeutic candidate to treat complex injuries such as MI/R injury. Moreover, exosomes are significantly stable in culture, allowing for their large quantity collection from a culture medium through their release over periods of time. Thus it is possible to develop scalable systems for exosome production. Packed-bed reactors and hollow-fiber bioreactors (HFBR) have been employed in biotechnology for years, and their utility in the efficient and scalable production of exosomes has recently been reported (51). They provide an extensive growth surface area supporting a large numbers of cells at high densities and allow the secreted exosomes to be collected from the reactor perfusate. In some applications collection of secreted products can be maintained over several months of continuous production.

Following their harvest or collection, by employing one or more distinct properties or characteristics of exosomes, including their size, density, morphology, composition, zeta potential, biochemical or immunological feature, these nano-sized particles are purified. Methods successfully employed include velocity and density gradients, agglomeration or precipitation via volume-excluding polymers, such as PEG or identified peptides, and a number of commercially available kits employing such proprietary means as active-ligand coated beads for adsorption chromatography (52). They can also be stored without potentially toxic cryopreservatives at -20°C for six months with no loss to their biochemical activity. In addition, much the same with conventional pharmaceutical products drug, exosomes can be tested in terms of dosage and biological activity.

However, demand for highly advanced purification and characterization technologies and significantly variable content of exosomes are two of the main obstacles in developing and utilization of exosomes in the context of regenerative medicine.

## Therapeutic effects of MSC-EXs in CVD

### *Anti-apoptotic effects*

Reduced ATP production, increased oxidative stress and apoptosis all are key features of cell death after myocardial ischemia/reperfusion (I/R) injury. Under this circumstance the lack of oxygen disrupts aerobic glycolysis and ATP production, leaving anaerobic glycolysis as the major source of ATP production. Reduced ATP production is a key feature of cardiac cell death during myocardial ischemic injury. As MSC-EXs were found to contain all five enzymes required for the ATP-generating phase of glycolysis, the investigators hypothesized that MSC-EX can increase ATP production in CMCs and attenuate their post-ischemic apoptosis. In line with this hypothesis, Lai et al. (53, 54) demonstrated that exosome treatment restored energy depletion, leading to limit cell death after myocardial I/R injury. Moreover, it was shown that MSC-EXs carry active CD73, an adenosine-generating extracellular enzyme, on their surface, which could activate adenosine receptors and induce Akt/GSK-3β survival kinase signaling pathway in CMCs (55, 56). Furthermore, peroxiredoxins and glutathione S-transferases, present in MSC-EXs, resulted in decrease of oxidative stress, a crucial cause of cardiac cell death. Altogether, available studies suggested that by inhibition of apoptosis via ameliorating ATP depletion and stimulation of survival pathways, MSC-EXs provide a short time window of opportunity for the cardiac cells to retrieve other molecular dysfunction leading to cardioprotection benefits.

A recent study conducted by Feng et al. (57) revealed that preconditioned murine BM-MSCs release exosomes enriched with miRNA-22 (miR-22). When CMCs were co-cultured with these MSCs, these released exosomes were internalized within the CMCs and delivered their miR-22 cargo. Subsequently, interaction of miR-22 with methyl CpG binding protein 2 (Mecp2), which reported to be elevated in cardiac abnormalities, resulted in protection of CMCs (58).

Another study carried out by Yu et al (59), exhibited that exosomes derived from rat BM-MSCs transduced with GATA-4, contained high levels of several miRNAs, including miR-221 and miR-19a. These exosomes were able to reduce apoptosis of ischemic CMCs via miR-221-dependent inhibition of p53-upregulated modulator of apoptosis (PUMA), a subclass of the Bcl-2 protein family. Furthermore, miR-19a cargo inhibited phosphatase and tensin homolog (PTEN), an inhibitor of the AKT survival signaling pathway, that resulted in activation of this survival pathway (60).

### *Anti-inflammatory effects*

Apoptosis of CMCs during ICDs activates an inflammatory response that serves to clean up the injury site from dead cells and stimulate the repair off damaged tissue. However, this response may go far beyond and even extend the injury. Thus inhibition of inflammation response is considered as a therapeutic approach for this condition. MSC-EXs are known to participate in inhibition of inflammatory response (61). In their study Lee et al (61) reported that the MSC-EXs inhibits lung inflammation in hypoxic pulmonary hypertension (HPH), leading to relieving symptoms and reversing HP. In the context of ICD, administration of MSC-EXs in ischemic/reperfused myocardium leaded to reduction of local and systemic inflammation which accompanied by reduced infarct size and improved cardiac function (55). Thus, inhibition of inflammation reactions suggested being involved in protective effects against.

### *Anti-cardiac remodeling*

Cardiac remodeling is a compensatory consequence of ICDs by which the remaining myocardium adapts to maintain cardiac function in the acute phase of injury. Remodeling encompasses cellular changes including CMCs hypertrophy, necrosis, apoptosis and fibrosis (62). In their study, Arslan et al. (55) indicated that the MSC-EXs are able to prevent adverse cardiac remodeling after myocardial I/R injury and MI via activating pro-survival signaling pathways, enhancing myocardial viability, restoring bioenergetics and reducing oxidative stress.

### *Angiogenesis*

Over a period of time, coronary collateral vessels and microvascular angiogenesis develop as a response to myocardial ischemia. It is thought that angiogenesis helps preserve the functionality of ischemic myocardium. Therapeutic coronary angiogenesis and collateralization have tremendous potential as treatment strategies for patients with ischemic heart disease.

This is because the biochemical cascades required for cell survival that are initiated by cells during no flow and ischemia are not compatible with the rapid restoration of flow and oxygen supply, and at the same time, cells cannot alter their biochemical activities expeditiously enough to adapt to this restoration. Cardiac repair and the improvement of cardiac function are known to be related to neovascularization, which involves arteriogenesis, vasculogenesis and angiogenesis.

Angiogenesis involves a variety of coordinated events including degradation of the extracellular matrix (63) surrounding the parent vessel, migration and proliferation of the endothelial cells and mural cells to assemble the new vessel, lumen formation, and construction of the mural cell layer of the vessel wall with associated pericytes and/or smooth muscle cells

Neovascularization is associated with proliferation and migration of endothelial cells (ECs) and VSMCs following by formation of tube-like structures. Exosomes from placental MSCs, induced by hypoxia, are reported to promote ECs migration and tube formation (64), which is contributed to placental vascular adaptation to low oxygen tension. In a recent study, performed by Kang et al. (65) administration of MSC-EXs overexpressing CXCR4 reported to promote angiogenesis and subsequent cardiac function improvement in rat MI model. Furthermore, Zhao et al. (43) demonstrated that intravenous administration of human umbilical MSC-EXs exosomes (hucMSC-EXs) following MI injury result in promotion of the tube formation and migration of ECs which is potentially related to myocardial repair and the improvement of cardiac function (43).

## Conclusion

Myocardial infarction is a leading cause of death among all cardiovascular diseases. Within the field of regenerative medicine, many have sought to use stem cells as a promising way to heal human tissue; however, in the past few years, cell-free regenerative medicine has quickly evolved to become a reality for therapeutic use in patients. Exosomes (packaged nano-vesicles released from cells) have shown exciting promise in this field. Identifying MSC-EXs as major mediators of therapeutic and paracrine effects of MSCs offers a rationale for employment of these cell-derived nanovesicles to replace viable cell transplantation. Exosomes can transport and deliver a large cargo of proteins, lipids, and nucleic acids and can modify cell and organ function. Much attention has been devoted to exploring the possible therapeutic roles of MSC-EXs in ICDs since they were discovered. Administration of these exosomes, which packaged with particular regenerative products, has showed promising cardiac regenerative and cardioprotective properties. Although a number of molecular mechanisms of exosomal-mediated cardiac repair are suggested, further investigation is required to fully understand the precise underlying mechanisms of these extremely encouraging nanovesicles. Future works will undoubtedly shed more light on the properties and functions of these natural carriers of biological molecules, paving the way for novel and exciting possibilities for the use of them in regenerative medicine including cardiac regeneration.

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## References

1. Wong ND. Epidemiological studies of CHD and the evolution of preventive cardiology. Nat Rev Cardiol 2014,11:276-289.
2. Gallina C, Turinetto V, Giachino C. A New Paradigm in Cardiac Regeneration: The Mesenchymal Stem Cell Secretome. Stem Cells Int 2015,2015:765846.
3. Bollini S, Smart N, Riley PR. Resident cardiac progenitor cells: at the heart of regeneration. J Mol Cell Cardiol 2011,50:296-303.
4. Gimble JM, Katz AJ, Bunnell BA. Adipose-derived stem cells for regenerative medicine. Circ Res 2007,100:1249-1260.
5. Wen Z, Zheng S, Zhou C, Yuan W, Wang J, Wang T. Bone marrow mesenchymal stem cells for post-myocardial infarction cardiac repair: microRNAs as novel regulators. J Cell Mol Med 2012,16:657-671.
6. Chullikana A, Majumdar AS, Gottipamula S, Krishnamurthy S, Kumar AS, Prakash VS, Gupta PK. Randomized, double-blind, phase I/II study of intravenous allogeneic mesenchymal stromal cells in acute myocardial infarction. Cytotherapy 2015,17:250-261.
7. Karantalis V, Hare JM. Use of mesenchymal stem cells for therapy of cardiac disease. Circ Res 2015,116:1413-1430.
8. Miyahara Y, Nagaya N, Kataoka M, Yanagawa B, Tanaka K, Hao H, Ishino K, Ishida H, Shimizu T, Kangawa K, Sano S, Okano T, Kitamura S, Mori H. Monolayered mesenchymal stem cells repair scarred myocardium after myocardial infarction. Nat Med 2006,12:459-465.
9. Williams AR, Hare JM. Mesenchymal stem cells: biology, pathophysiology, translational findings, and therapeutic implications for cardiac disease. Circ Res 2011,109:923-940.
10. Premer C, Blum A, Bellio MA, Schulman IH, Hurwitz BE, Parker M, Dermarkarian CR, DiFede DL, Balkan W, Khan A, Hare JM. Allogeneic Mesenchymal Stem Cells Restore Endothelial Function in Heart Failure by Stimulating Endothelial Progenitor Cells. EBioMedicine 2015,2:467-475.
11. Iso Y, Spees JL, Serrano C, Bakondi B, Pochampally R, Song YH, Sobel BE, Delafontaine P, Prockop DJ. Multipotent human stromal cells improve cardiac function after myocardial infarction in mice without long-term engraftment. Biochem Biophys Res Commun 2007,354:700-706.
12. Leiker M, Suzuki G, Iyer VS, Canty JM, Jr., Lee T. Assessment of a nuclear affinity labeling method for tracking implanted mesenchymal stem cells. Cell Transplant 2008,17:911-922.
13. Gnecchi M, He H, Liang OD, Melo LG, Morello F, Mu H, Noiseux N, Zhang L, Pratt RE, Ingwall JS, Dzau VJ. Paracrine action accounts for marked protection of ischemic heart by Akt-modified mesenchymal stem cells. Nat Med 2005,11:367-368.
14. Khan M, Nickoloff E, Abramova T, Johnson J, Verma SK, Krishnamurthy P, Mackie AR, Vaughan E, Garikipati VN, Benedict C, Ramirez V, Lambers E, Ito A, Gao E, Misener S, Luongo T, Elrod J, Qin G, Houser SR, Koch WJ, Kishore R. Embryonic Stem Cell-Derived Exosomes Promote Endogenous Repair Mechanisms and

Enhance Cardiac Function Following Myocardial Infarction. Circ Res 2015,117:52-64.
15. Xin H, Li Y, Cui Y, Yang JJ, Zhang ZG, Chopp M. Systemic administration of exosomes released from mesenchymal stromal cells promote functional recovery and neurovascular plasticity after stroke in rats. J Cereb Blood Flow Metab 2013,33:1711-1715.
16. Caplan AI, Dennis JE. Mesenchymal stem cells as trophic mediators. J Cell Biochem 2006,98:1076-1084.
17. Rehman J, Traktuev D, Li J, Merfeld-Clauss S, Temm-Grove CJ, Bovenkerk JE, Pell CL, Johnstone BH, Considine RV, March KL. Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells. Circulation 2004,109:1292-1298.
18. Fazel S, Chen L, Weisel RD, Angoulvant D, Seneviratne C, Fazel A, Cheung P, Lam J, Fedak PW, Yau TM, Li RK. Cell transplantation preserves cardiac function after infarction by infarct stabilization: augmentation by stem cell factor. J Thorac Cardiovasc Surg 2005,130:1310.
19. Noiseux N, Gnecchi M, Lopez-Ilasaca M, Zhang L, Solomon SD, Deb A, Dzau VJ, Pratt RE. Mesenchymal stem cells overexpressing Akt dramatically repair infarcted myocardium and improve cardiac function despite infrequent cellular fusion or differentiation. Mol Ther 2006,14:840-850.
20. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop D, Horwitz E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006,8:315-317.
21. Chong JJ, Chandrakanthan V, Xaymardan M, Asli NS, Li J, Ahmed I, Heffernan C, Menon MK, Scarlett CJ, Rashidianfar A, Biben C, Zoellner H, Colvin EK, Pimanda JE, Biankin AV, Zhou B, Pu WT, Prall OW, Harvey RP. Adult cardiac-resident MSC-like stem cells with a proepicardial origin. Cell Stem Cell 2011,9:527-540.
22. Amado LC, Saliaris AP, Schuleri KH, St John M, Xie JS, Cattaneo S, Durand DJ, Fitton T, Kuang JQ, Stewart G, Lehrke S, Baumgartner WW, Martin BJ, Heldman AW, Hare JM. Cardiac repair with intramyocardial injection of allogeneic mesenchymal stem cells after myocardial infarction. Proc Natl Acad Sci USA 2005,102:11474-11479.
23. Boyle AJ, McNiece IK, Hare JM. Mesenchymal stem cell therapy for cardiac repair. Methods Mol Biol 2010,660:65-84.
24. Zimmet JM, Hare JM. Emerging role for bone marrow derived mesenchymal stem cells in myocardial regenerative therapy. Basic Res Cardiol 2005,100:471-481.
25. Minguell JJ, Erices A. Mesenchymal stem cells and the treatment of cardiac disease. Exp Biol Med (Maywood) 2006,231:39-49.
26. Toma C, Pittenger MF, Cahill KS, Byrne BJ, Kessler PD. Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. Circulation 2002,105:93-98.
27. Quevedo HC, Hatzistergos KE, Oskouei BN, Feigenbaum GS, Rodriguez JE, Valdes D, Pattany PM, Zambrano JP, Hu Q, McNiece I, Heldman AW, Hare JM. Allogeneic mesenchymal stem cells restore cardiac function in chronic ischemic cardiomyopathy via trilineage differentiating capacity. Proc Natl Acad Sci USA 2009,106:14022-14027.
28. Dixon JA, Gorman RC, Stroud RE, Bouges S, Hirotsugu H, Gorman JH, 3rd, Martens TP, Itescu S, Schuster MD, Plappert T, St John-Sutton MG, Spinale FG. Mesenchymal cell transplantation and myocardial remodeling after myocardial infarction. Circulation 2009,120:S220-229.
29. Hatzistergos KE, Quevedo H, Oskouei BN, Hu Q, Feigenbaum GS, Margitich IS, Mazhari R, Boyle AJ, Zambrano JP, Rodriguez JE, Dulce R, Pattany PM, Valdes D, Revilla C, Heldman AW, McNiece I, Hare JM. Bone marrow mesenchymal stem cells stimulate cardiac stem cell proliferation and differentiation. Circ Res 2010,107:913-922.
30. Loffredo FS, Steinhauser ML, Gannon J, Lee RT. Bone marrow-derived cell therapy stimulates endogenous cardiomyocyte progenitors and promotes cardiac repair. Cell Stem Cell 2011,8:389-398.
31. Mazhari R, Hare JM. Mechanisms of action of mesenchymal stem cells in cardiac repair: potential influences on the cardiac stem cell niche. Nat Clin Pract Cardiovasc Med 2007,4 Suppl 1:S21-26.
32. Mirotsou M, Jayawardena TM, Schmeckpeper J, Gnecchi M, Dzau VJ. Paracrine mechanisms of stem cell reparative and regenerative actions in the heart. J Mol Cell Cardiol 2011,50:280-289.
33. Gyorgy B, Szabo TG, Pasztoi M, Pal Z, Misjak P, Aradi B, Laszlo V, Pallinger E, Pap E, Kittel A, Nagy G, Falus A, Buzas EI. Membrane vesicles, current state-of-the-art: emerging role of extracellular vesicles. Cell Mol Life Sci 2011,68:2667-2688.
34. Gnecchi M, He H, Noiseux N, Liang OD, Zhang L, Morello F, Mu H, Melo LG, Pratt RE, Ingwall JS, Dzau VJ. Evidence supporting paracrine hypothesis for Akt-modified mesenchymal stem cell-mediated cardiac protection and functional improvement. Faseb J 2006,20:661-669.
35. Timmers L, Lim SK, Arslan F, Armstrong JS, Hoefer IE, Doevendans PA, Piek JJ, El Oakley RM, Choo A, Lee CN, Pasterkamp G, de Kleijn DP. Reduction of myocardial infarct size by human mesenchymal stem cell conditioned medium. Stem Cell Res 2007,1:129-137.
36. Roy R, Brodarac A, Kukucka M, Kurtz A, Becher PM, Julke K, Choi YH, Pinzur L, Chajut A, Tschope C, Stamm C. Cardioprotection by placenta-derived stromal cells in a murine myocardial infarction model. J Surg Res 2013,185:70-83.
37. Lai RC, Arslan F, Lee MM, Sze NS, Choo A, Chen TS, Salto-Tellez M, Timmers L, Lee CN, El Oakley RM, Pasterkamp G, de Kleijn DP, Lim SK. Exosome secreted by MSC reduces myocardial ischemia/reperfusion injury. Stem Cell Res 2010,4:214-222.
38. Colombo M, Raposo G, Thery C. Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. Annu Rev Cell Dev Biol 2014,30:255-289.
39. Zhou Y, Xu H, Xu W, Wang B, Wu H, Tao Y, Zhang B, Wang M, Mao F, Yan Y, Gao S, Gu H, Zhu W, Qian H. Exosomes released by human umbilical cord mesenchymal stem cells protect against cisplatin-induced renal oxidative stress and apoptosis in vivo and in vitro. Stem Cell Res Ther 2013,4:34.
40. Shabbir A, Cox A, Rodriguez-Menocal L, Salgado M, Badiavas EV. Mesenchymal Stem Cell Exosomes Induce Proliferation and Migration of Normal and Chronic Wound Fibroblasts, and Enhance Angiogenesis In Vitro. Stem Cells Dev 2015,24:1635-1647.
41. Tan CY, Lai RC, Wong W, Dan YY, Lim SK, Ho HK. Mesenchymal stem cell-derived exosomes promote hepatic regeneration in drug-induced liver injury models. Stem Cell Res Ther 2014,5:76.
42. Zhang Y, Chopp M, Meng Y, Katakowski M, Xin H, Mahmood A, Xiong Y. Effect of exosomes derived from multipluripotent mesenchymal stromal cells on functional recovery and neurovascular plasticity in rats after traumatic brain injury. J Neurosurg 2015,122:856-867.
43. Zhao Y, Sun X, Cao W, Ma J, Sun L, Qian H, Zhu W, Xu W. Exosomes Derived from Human Umbilical Cord Mesenchymal Stem Cells Relieve Acute Myocardial Ischemic Injury. Stem Cells Int 2015,2015:761643.
44. Poh KK, Sperry E, Young RG, Freyman T, Barringhaus KG, Thompson CA. Repeated direct endomyocardial transplantation of allogeneic mesenchymal stem cells: safety of a high dose, "off-the-shelf", cellular cardiomyoplasty strategy. Int J Cardiol 2007,117:360-364.
45. Furlani D, Ugurlucan M, Ong L, Bieback K, Pittermann E, Westien I, Wang W, Yerebakan C, Li W, Gaebel R, Li RK, Vollmar B, Steinhoff G, Ma N. Is the intravascular administration of mesenchy-

mal stem cells safe? Mesenchymal stem cells and intravital microscopy. Microvasc Res 2009,77:370-376.
46. Hunt DP, Irvine KA, Webber DJ, Compston DA, Blakemore WF, Chandran S. Effects of direct transplantation of multipotent mesenchymal stromal/stem cells into the demyelinated spinal cord. Cell Transplant 2008,17:865-873.
47. Parolini I, Federici C, Raggi C, Lugini L, Palleschi S, De Milito A, Coscia C, Iessi E, Logozzi M, Molinari A, Colone M, Tatti M, Sargiacomo M, Fais S. Microenvironmental pH is a key factor for exosome traffic in tumor cells. J Biol Chem 2009,284:34211-34222.
48. Murphy E, Steenbergen C. Mechanisms underlying acute protection from cardiac ischemia-reperfusion injury. Physiol Rev 2008,88:581-609.
49. Kukielka GL, Hawkins HK, Michael L, Manning AM, Youker K, Lane C, Entman ML, Smith CW, Anderson DC. Regulation of intercellular adhesion molecule-1 (ICAM-1) in ischemic and reperfused canine myocardium. J Clin Invest 1993,92:1504-1516.
50. Rieu S, Geminard C, Rabesandratana H, Sainte-Marie J, Vidal M. Exosomes released during reticulocyte maturation bind to fibronectin via integrin alpha4beta1. Eur J Biochem 2000,267:583-590.
51. Bsargent. Exosomes: A Major Phenomenon Flying Under the Radar. The Cell Culture Dish, September 24, 2015.
52. Lane RE, Korbie D, Anderson W, Vaidyanathan R, Trau M. Analysis of exosome purification methods using a model liposome system and tunable-resistive pulse sensing. Sci Rep 2015,5:7639.
53. Lai RC, Yeo RWY, Tan SS, Zhang B, Yin Y, Sze NSK, Choo A, Lim SK. Mesenchymal stem cell exosomes: the future msc-based therapy? Mesenchymal Stem Cell Therapy: Springer, 2013:39-61.
54. Lai RC, Yeo RW, Tan KH, Lim SK. Mesenchymal stem cell exosome ameliorates reperfusion injury through proteomic complementation. Regen Med 2013,8:197-209.
55. Arslan F, Lai RC, Smeets MB, Akeroyd L, Choo A, Aguor EN, Timmers L, van Rijen HV, Doevendans PA, Pasterkamp G, Lim SK, de Kleijn DP. Mesenchymal stem cell-derived exosomes increase ATP levels, decrease oxidative stress and activate PI3K/Akt pathway to enhance myocardial viability and prevent adverse remodeling after myocardial ischemia/reperfusion injury. Stem Cell Res 2013,10:301-312.
56. Li X, Arslan F, Ren Y, Adav SS, Poh KK, Sorokin V, Lee CN, de Kleijn D, Lim SK, Sze SK. Metabolic adaptation to a disruption in oxygen supply during myocardial ischemia and reperfusion is underpinned by temporal and quantitative changes in the cardiac proteome. J Proteome Res 2012,11:2331-2346.
57. Feng Y, Huang W, Wani M, Yu X, Ashraf M. Ischemic preconditioning potentiates the protective effect of stem cells through secretion of exosomes by targeting Mecp2 via miR-22. PLoS One 2014,9:e88685.
58. Alvarez-Saavedra M, Carrasco L, Sura-Trueba S, Demarchi Aiello V, Walz K, Neto JX, Young JI. Elevated expression of MeCP2 in cardiac and skeletal tissues is detrimental for normal development. Hum Mol Genet 2010,19:2177-2190.
59. Yu B, Gong M, Wang Y, Millard RW, Pasha Z, Yang Y, Ashraf M, Xu M. Cardiomyocyte protection by GATA-4 gene engineered mesenchymal stem cells is partially mediated by translocation of miR-221 in microvesicles. PLoS One 2013,8:e73304.
60. Yu B, Kim HW, Gong M, Wang J, Millard RW, Wang Y, Ashraf M, Xu M. Exosomes secreted from GATA-4 overexpressing mesenchymal stem cells serve as a reservoir of anti-apoptotic microRNAs for cardioprotection. Int J Cardiol 2015,182:349-360.
61. Lee C, Mitsialis SA, Aslam M, Vitali SH, Vergadi E, Konstantinou G, Sdrimas K, Fernandez-Gonzalez A, Kourembanas S. Exosomes mediate the cytoprotective action of mesenchymal stromal cells on hypoxia-induced pulmonary hypertension. Circulation 2012,126:2601-2611.
62. Cohn JN, Ferrari R, Sharpe N. Cardiac remodeling--concepts and clinical implications: a consensus paper from an international forum on cardiac remodeling. Behalf of an International Forum on Cardiac Remodeling. J Am Coll Cardiol 2000,35:569-582.
63. Vrijsen KR, Sluijter JP, Schuchardt MW, van Balkom BW, Noort WA, Chamuleau SA, Doevendans PA. Cardiomyocyte progenitor cell-derived exosomes stimulate migration of endothelial cells. J Cell Mol Med 2010,14:1064-1070.
64. Salomon C, Ryan J, Sobrevia L, Kobayashi M, Ashman K, Mitchell M, Rice GE. Exosomal signaling during hypoxia mediates microvascular endothelial cell migration and vasculogenesis. PLoS One 2013,8:e68451.
65. Kang K, Ma R, Cai W, Huang W, Paul C, Liang J, Wang Y, Zhao T, Kim HW, Xu M, Millard RW, Wen Z. Exosomes Secreted from CXCR4 Overexpressing Mesenchymal Stem Cells Promote Cardioprotection via Akt Signaling Pathway following Myocardial Infarction. Stem Cells Int 2015,2015:659890.