Myocardial infarction (MI), a common presentation for ischemic heart disease/coronary artery disease, is a leading cause of death worldwide. Twenty million people die from cardiovascular diseases each year, and in the United States alone, 1.5 million people per year have MI. Patients who survive are at high risk of recurrent MI and heart failure. From 1990 to 2009, coronary heart disease (including acute MI and angina) was the leading cause of death (death from acute MI was the highest), with the highest economic cost of all major disease categories. Although there are treatments to mitigate the initial cardiac damage during an acute MI, there is a need for novel treatments to minimize subsequent cardiac remodeling that can adversely affect heart function. In this context, identifying new targets to improve tissue repair, including preservation of the cardiac microvasculature, clearance of apoptotic cells, and tissue regeneration, are of great interest.

Exosomes are small membrane-bound vesicles (30–100 nm) of endocytic origin, actively secreted by most cell types. They are derived from the luminal membranes of multivesicular bodies and constitutively released by fusion of multivesicular bodies with the cell membrane. Exosomes can mediate cellular-, tissue-, and organ-level microcommunication under normal and pathological conditions by shuttling proteins, mRNA, and microRNAs (miRNAs).

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In this review, we critically examine the emerging role of exosomes in local and distant microcommunication mechanisms after MI. A comprehensive understanding of the role of exosomes in cardiac repair after myocardial infarction could bridge a major gap in knowledge of the repair mechanism after myocardial injury. (Circ Res. 2014;114:333-344.)

Key Words: adult stem cells ■ exosomes ■ microRNAs ■ multivesicular bodies ■ myocardial infarction ■ progenitor cells

Exosomes and Cardiac Repair After Myocardial Infarction
Susmita Sahoo, Douglas W. Losordo

Abstract: Myocardial infarction is a leading cause of death among all cardiovascular diseases. The analysis of molecular mechanisms by which the ischemic myocardium initiates repair and remodeling indicates that secreted soluble factors are key players in communication to local and distant tissues, such as bone marrow. Recently, actively secreted membrane vesicles, including exosomes, are being recognized as new candidates with important roles in intercellular and tissue-level communication. In this review, we critically examine the emerging role of exosomes in local and distant microcommunication mechanisms after myocardial infarction. A comprehensive understanding of the role of exosomes in cardiac repair after myocardial infarction could bridge a major gap in knowledge of the repair mechanism after myocardial injury. (Circ Res. 2014;114:333-344.)

Key Words: adult stem cells ■ exosomes ■ microRNAs ■ multivesicular bodies ■ myocardial infarction ■ progenitor cells

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In this review, we critically examine the emerging role of exosomes in local and distant microcommunication mechanisms after MI. In the first part, we recapitulate the regeneration mechanism of the mammalian heart in the light of current literature. Then, we discuss evidence that post-MI the myocardium secretes exosomes that could carry circulating miRNAs. We conclude that a comprehensive study of the role of exosomes in cardiac repair after MI could bridge a major gap in knowledge as well as accelerate our understanding of the
mechanism of cell therapies. This new knowledge could be used to refine existing treatments and to develop alternative therapeutic approaches that may benefit patients with cardiovascular diseases.

Cardiac Repair and Regeneration After MI: What Is Known?

Endogenous Repair
It is now well established that the human heart is a dynamic organ capable of continuous regeneration, the degree of replacement now being the only subject of debate.4,7 The human left ventricle has 2 to 4 billion cardiomyocytes, and a MI can destroy ≥25% of the myocyte population in a few hours.5,8,9 Because of the heart’s limited ability to regenerate rapidly after a catastrophic insult, such as a MI, scar formation, rather than muscle regeneration, is often a major component of the healing response. The regeneration/proliferation of cardiomyocytes may be faster in the border zone of an injured heart as compared with the normal heart,10 both in mice11 and in humans.7 Nevertheless, the limited endogenous reparative mechanisms in the adult mammalian heart seem to depend more on the replenishment by progenitor cells than on replacement by cardiomyocyte proliferation.12 Several reports suggest that acute cardiac injury involves the activation and recruitment of both resident cardiac progenitor cells and noncardiac progenitor cells from BM that home in to the site of injury.13–18 In the absence of administration of external growth factors or progenitor cell therapies, the endogenous regenerative process is insufficient in a significant plurality, as evidenced by factors or progenitor cell therapies, the endogenous regenerative function now being the only subject of debate.4–7 The human left ventricle has 2 to 4 billion cardiomyocytes, and a MI can destroy ≥25% of the myocyte population in a few hours.5,8,9 Because of the heart’s limited ability to regenerate rapidly after a catastrophic insult, such as a MI, scar formation, rather than muscle regeneration, is often a major component of the healing response. The regeneration/proliferation of cardiomyocytes may be faster in the border zone of an injured heart as compared with the normal heart,10 both in mice11 and in humans.7 Nevertheless, the limited endogenous reparative mechanisms in the adult mammalian heart seem to depend more on the replenishment by progenitor cells than on replacement by cardiomyocyte proliferation.12 Several reports suggest that acute cardiac injury involves the activation and recruitment of both resident cardiac progenitor cells and noncardiac progenitor cells from BM that home in to the site of injury.13–18 In the absence of administration of external growth factors or progenitor cell therapies, the endogenous regenerative process is insufficient in a significant plurality, as evidenced by factors or progenitor cell therapies, the endogenous regenerative process is insufficient in a significant plurality, as evidenced by the occurrence of post-MI heart in ≥30% of patients.19 Moreover, experimental studies have suggested the release of stem and progenitor cells into the circulation from BM niche, which could play an important role in the turnover of vascular endothelium and myocardial repair response after MI.20

Mobilization and Homing of BM Progenitor Cells
Numerous studies have confirmed that MI induces rapid mobilization of hematopoietic stem cells, endothelial progenitor cells, mesenchymal stromal cells, circulating angiogenic cells, and pluripotent small embryonic-like cells from BM, although there is no consensus on the identification criteria used to identify different populations of progenitor cells. The mobilization of BM-derived stem cells (BM-SCs) in patients with acute MI is considered a reparatory response,21–24 and experimental studies have suggested an important role of BM-SCs in cardiac repair response after MI.13,20,24–29 Nevertheless, the contribution of circulating cells to myocardial and endothelial repair is still incompletely understood. Moreover, the mobilization of endothelial progenitor cells correlates with the ischemic zone and not with the necrotic zone of patients after MI,30 suggesting the possibility of an active mechanism originating from the ischemic border zone of the heart that is transmitted to the BM.

Mechanism of Mobilization
In acute MI, the systemic levels of soluble inflammatory mediators, chemokines, cytokines, and growth factors are significantly increased, some of which act as chemoattractants to BM-SCs.20,31 It is possible that ischemic tissue releases these chemoattractants, which create a gradient directing the cells to the site of injury. This mechanism was aptly demonstrated in vitro by positive migration of BM-SCs expressing early cardiac markers toward the homogenates of infarcted mouse myocardium enriched with stromal-derived factor-1, hepatocyte growth factor, and leukemia inhibitory factor.31 The complete mechanism of BM-SC mobilization is likely much more complicated because, first, the plasma levels of chemokines (stromal-derived factor-1) are not correlated with the extent of BM-SC mobilization20; second, the measurement of plasma levels does not necessarily reflect local concentrations in the BM and ischemic tissue, which is more important for cell egress and homing; and third, as the recent evidence suggests, other signaling pathways and mechanisms, including exosomes and miRNAs, could promote trafficking and engraftment of BM and cardiac SCs, as we will discuss later.

Nonetheless, the existence of small but measurable amounts of local and distant stem and progenitor cells in an injured heart implies that the endogenous regenerative potential could be amplified by therapeutic interventions restoring contractile function in scar areas.

Augmentation of Cardiac Repair With Cell Therapies
Multiple experimental and several clinical studies have shown that different subsets of BM-derived cells, isolated either from BM or from peripheral blood, and other adult progenitor cells isolated from the cardiac tissue improved the recovery of heart function.32–38 Although the degree of improvement in cardiac function varies depending on the target population and cell type,35 there is mounting evidence that cell therapy is safe and does have a beneficial effect on LV function39 that translates into clinical benefit.7 One of the most important questions concerning cell therapies is to elucidate the mechanism by which stem/progenitor cells achieve functional improvement: do cell therapies function via protection of the existing functional tissue or by regenerating new heart tissue, or both? If regeneration occurs, does this result from the direct contribution of transplanted cells to form new tissue, paracrine stimulation of local and remote stem/progenitor cells or, most likely, in our opinion, a combination of these mechanisms, particularly in the case of autologous therapies? Most cell therapies show evidence for the stimulation of neovascularization and proliferation of endothelial cells essential for new capillary formation. Evidence for neomyogenesis is less well developed. However, cardiac stem cell and cardiac/cardiospheres-derived progenitor cells have been shown to enhance cardiomyocyte proliferation as well as myocyte replenishment.40

Evidence from preclinical and clinical studies involving cell-based therapies encountered a disproportionate benefit of cellular grafts in the heart; a modest number of retained cells in

<table>
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<th>Nonstandard Abbreviations and Acronyms</th>
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<tr>
<td>BM bone marrow</td>
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<tr>
<td>BM-SC BM-derived stem cell</td>
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<tr>
<td>EV extracellular vesicle</td>
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<tr>
<td>MI myocardial infarction</td>
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<td>MVE multivesicular endosome</td>
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Evidence from preclinical and clinical studies involving cell-based therapies encountered a disproportionate benefit of cellular grafts in the heart; a modest number of retained cells in
the myocardium appeared to account for marked improvements in cardiac function.\textsuperscript{41} These observations have raised the possibility that grafted cells may amplify their effects by producing growth factors, cytokines, or other signaling entities that improve the performance or survival of resident or recruited cells. Several studies have suggested that a paracrine mechanism contributes significantly to myocardial repair triggered by cell-based therapies (reviewed here).\textsuperscript{42–44} Neovascularization and neomyogenesis can be mediated by progenitor cells, which release factors to modulate the microenvironment and to act on resident stem/progenitor or mature cells. These factors can also beneficially influence cardiac repair by protecting cardiac myocytes from apoptotic stimuli. Collectively, these studies indicate that the paracrine effects of progenitor cells could be an important mechanism of cell therapy.

**Exosomes as a Potential Intercellular Communicator of Ischemic Signaling and Myocardial Repair**

Cells communicate with each other via extracellular molecules, such as nucleotides, lipids, short peptides, or proteins. These molecules are released extracellularly by cells and bind to receptors on other cells, thus inducing intracellular signaling and modification of the intracellular physiological state of recipient cells. In addition to these single molecules, eukaryotic cells also release complex structures called membrane vesicles, which are a rich source of numerous proteins, lipids, and nucleic acids that can affect cells that encounter these structures in much more complex ways.\textsuperscript{3} Most of the extracellular and body fluids, such as blood, urine, saliva, and breast milk, are known to contain secreted vesicles, called microvesicles and exosomes. Although known for several decades, extracellular membrane vesicles have long been thought of as mere cell debris, signs of cell death, or structures specific to a unique organ. The importance of exosomes and the recognition of its wider involvement in intercellular signaling began to emerge from this picture in the last decade. Growing attention is now being focused on exosome-mediated cell–cell communication, a mechanism that has been largely overlooked previously.

Extracellular vesicles (EVs) are broadly subdivided into different categories, depending on their origin, size, morphology, and method of vesicle collection\textsuperscript{45,46} (discussed at the annual meeting of International Society for Extracellular Vesicles, 2012, session on vesicle nomenclature). Mounting evidence suggests that several cell types used in regenerative medicine secrete some form of EVs, including microvesicles and exosomes. Exosomes derived from stem cells have recently been recognized in the stimulation of angiogenesis and cytoprotection and modulation of inflammation and apoptosis.\textsuperscript{51,47,48} More importantly, EVs, which are present in abundance in blood plasma, are a rich source of circulating miRNAs. Several recent studies have demonstrated that the plasma levels of circulating miRNAs are altered in patients after MI and that these circulating miRNAs can be used as biomarkers to detect MI in patients.\textsuperscript{59–52} However, the specific cellular source of miRNAs, mechanism of miRNA release after ischemia, and whether the released miRNAs are sheltered inside EVs are still not clear. Interestingly, cardiomyocytes, which were initially considered as not-so-secretory cell type, have now been suggested to secrete exosomes.\textsuperscript{49,53,54}

In this context, the investigation of the role of exosomes in myocardial remodeling and repair is potentially important. Exosomes may provide the underlying mechanisms by which the damaged heart communicates with other tissues and organs to initiate the repair process, and how stem/progenitor cells repair and regenerate the myocardium. The study of exosomes from ischemic heart may reveal important cell–cell communication and signaling mechanisms for local and distant tissues such as BM.

**Biology of Exosomes**

Exosomes represent a specific subset of secreted membrane vesicles, which are relatively homogeneous in size (30–100 nm). Exosomes have been proposed to differ from other membrane vesicles by its size, density, and specific composition of lipids, proteins, and nucleic acids, which reflect its endocytic origin.\textsuperscript{55}

**Biogenesis and Secretion of Exosomes**

Exosomes are formed in endosomal vesicles called multivesicular endosomes (MVEs) or multivesicular bodies, which originate by direct budding of the plasma membrane into early endosomes. The generation of exosomes to form MVEs involves the lateral segregation of cargo at the delimiting membrane of an endosome and inward budding and pinching of vesicles into the endosomal lumen. Because exosomes originate by 2 successive invaginations from the plasma membrane, its membrane orientation is similar to the plasma membrane (Figure 1). Exosomes from many cell types may contain similar surface proteins as the cell from which it is derived.\textsuperscript{47} Membrane proteins that are known to cluster into microdomains at the plasma membrane or at endosomes, such as tetraspanins (CD63, CD81, CD82), often are also enriched in EVs. It is also thought that endosomal sorting complex responsible for transport system and tetraspanins, which are highly enriched in MVEs, play a role in exosome production.\textsuperscript{45} How cytosolic constituents are recruited into exosomes is unclear but may involve the association of exosomal membrane proteins with chaperones, such as HSC70, that are found in exosomes from most cell types. MVEs are also sites of miRNA-loaded RNA-induced silencing complex accumulation,\textsuperscript{56} and the fact that exosome-like vesicles are considerably enriched in GW182 and AGO2 implicates the functional roles of these proteins in RNA sorting to exosomes. Exosomes are released to the extracellular fluid by fusion of MVE to the plasma membrane of a cell, resulting in bursts of exosome secretion. Several Rab GTPases such as Rab 27a and Rab27b,\textsuperscript{57} Rab11 and Rab35, all seem to be involved in exosomes release.\textsuperscript{45}

Exosomes are secreted by multiple cell types and cell lines, including stem cells, endothelial cells, smooth muscle cells, neuronal cells, and tumor cells,\textsuperscript{47,58–60} and are detected in most body fluids, such as blood, urine, saliva, cerebrospinal fluid, and ascites.\textsuperscript{45}

**Isolation and Characterization Techniques**

Exosomes can be isolated from any biological fluid it is secreted to—either from the body fluids or from the supernatant of cells grown in a media free of exosomes (because serum contains exosomes, the media should be serum-free or exosomes depleted by ultracentrifugation of serum).\textsuperscript{61} Experimental procedures...
used to purify exosomes are mostly based on its unique size and density. The most rigorous and accepted protocols involve clearing the sample fluid by sequential centrifugations and pelleting the exosomes at 100,000 to 110,000 g, followed by resuspensions and repelleting. For purer preparations, a sucrose gradient is used, because exosomes float at a density that ranges from 1.13 to 1.19 g/mL. Recently, several alternate methods, such as microfluidic devices, antibody-coated magnetic beads, and precipitation-based, filtration-based isolation, have been developed and are reviewed here in detail.

Initial characterization of exosomes is typically based on electron microscopy analysis because their size, <100 nm, is below the resolution of a light microscope. Beside electron microscopy, both dynamic light scattering analysis and nanoparticle tracking analysis allow the determination of the size of exosomes; nanoparticle tracking analysis also allows the determination of concentration. Further characterization of isolated exosomes requires complementary biochemical (immunoblotting and flowcytometry), mass spectrometry, and imaging techniques.

Molecular Composition of Exosomes

Exosomes have been shown to carry a unique cargo of lipids, proteins, and RNAs, which are often distinct from the cell of its origin. Because of its endosomal origin, all exosomes contain membrane transport and fusion proteins (GTPases, annexins, flotillin), tetraspannins (CD9, CD63, CD81, CD82), heat shock proteins (HSC70, HSP90), proteins involved in multivesicular body biogenesis (Alix, TSG101), as well as lipid-related proteins and phospholipases. The molecular lipid composition of exosomes is largely unknown, although exosomes show a remarkable enrichment of distinct lipids such as glycosphingolipids, sphingomyelin, cholesterol, and phosphatidylserine as compared with the cell of its origin. An important breakthrough in understanding the biological significance of exosomes came from the finding that exosomes harbor a cargo of functional mRNAs and miRNAs, a discovery that has opened up new avenues of research on the role of exosomes in cellular crosstalk. Interestingly, exosomes contain a select subset of cellular RNAs or completely distinct RNAs, some of which are tissue-specific, whereas others are ubiquitous to all exosomes regardless of its cell of origin. A specific targeting of RNA sequences is thought to occur at the time of exosome biogenesis, thereby refuting the idea that RNAs in exosomes result from a random contamination of secreted vesicles by RNAs released extracellularly from dying cells. The presence of DNA in exosomes is controversial. At present, it is not clear whether the DNA found in isolated exosomes is functional. Limited number of reports that describe exosomal DNA did not rule out the presence of viral DNAs in incorporated or isolated along with exosomal preparations, or whether it could be a result of contamination from dead cells in culture.

Exosomes Act as a Vector for Intercellular and Tissue-Level Microcommunication

Once secreted, exosomes either interact with surrounding cells or can be released to the systemic circulation; they are even shown to cross the blood–brain barrier. Many fluorescence microscopy studies demonstrated the capturing and accumulation of these vesicles in internal endocytic or phagocytic compartments (especially in phagocytic cells such as macrophages). (Note that because exosomes are <200 nm in diameter, individual vesicles cannot be detected by confocal microscopy techniques; they are visible once the fluorescent-tagged vesicles accumulate intracellularly. Only high-resolution electron microscopy allows for the visualization of individual exosomes.) Exosomes from a specific cell of origin can selectively bind and be internalized by certain target cell types and not by others, although the cellular and molecular basis for this targeting is still undetermined. Furthermore, precise mechanisms
by which individual exosomes interact with recipient cells are also not clear. It has been proposed that exosomes bind to the plasma membrane of recipient cells via specific receptors and are either internalized by micropinocytosis to fuse with the membrane to release its contents of proteins, lipids, and RNAs to the cytosol, or are internalized by distinct endocytosis. When endocytosed, exosomes can subsequently fuse with the endosomal delimiting membrane or can be targeted to lysosomes for degradation. Exosomes can mediate local and systemic cell communication through the horizontal transfer of information, such as proteins, mRNAs, and miRNAs, and are known to induce physiological changes in recipient cells. Some of the important functions of exosomes include, but not limited to, tumor progression by promoting angiogenesis, tumor metastasis, acting as antigen-presenting vesicles to stimulate antitumoral responses, control tissue fibrosis, disseminate Alzheimer pathogenesis, and have been implicated in the therapeutic activities of stem cells.

In a landmark publication, Skog et al. demonstrated a role for both proteins and the RNA content of exosomes/microvesicles from cultured glioblastoma tumor cells in disseminating malignancy to recipient cells in the tumor microenvironment. Glioblastoma exosomes contain several angiogenic proteins and genetic information in the form of RNA, which are transferred to and translated by the recipient brain microvascular endothelial cells to induce angiogenic activity. Furthermore, a tumor-specific mRNA, EGFRvIII, was detected in serum exosomes of glioblastoma patients. This study suggests that proteins and RNA from tumor-derived exosomes can provide diagnostic information and aid in therapeutic decisions for cancer patients.

Several properties of exosomes make them interesting, suitable for ex vivo study and manipulation, and potentially potent in relation to intramyocardial or distant tissue communication:

1. Exosomes have a unique protein/miRNA composition, which in some cases differs from the parent cell of origin.
2. They have specific biophysical properties, such as size and density for floatation, which enable easy separation from contaminating debris and other vesicles.

Figure 2. Presence of double-membrane–bound exosomes in multivesicular bodies (MVBs) in cardiomyocytes from (A) healthy and (B) ischemic human heart. Frozen heart tissues from the left ventricle were processed for electron microscopy as described previously.
3. Exosomes have a unique rigid lipid membrane that makes them insensitive to freeze-thaw cycles and resistant to bursting in a hypotonic environment and facilitates efficient delivery to other cells.60,77

4. They exhibit cell-specific signaling (exosomes are suspected to have specific receptors for target cell signaling).

5. Exosomes seem to be capable of acting as vehicles for drug delivery with easy isolation and a potential for manipulation of the expression of RNA and protein contents.58

Myocardium Secretes Exosomes: In Vivo Evidence

Exosomes are demonstrated to be mediators of extracellular communication; therefore, it is fitting to propose that they can be important communicators of ischemic signaling and myocardial repair. Reports have suggested that the myocardial tissue secretes exosomes, and exosomes and microvesicles could be an important mechanism involved in heterocellular communication in the adult heart,78,79 especially exosomes emerging from telocytes in the border zone of MI.79 Barile et al79 provided ultrastructural evidence for the first time that exosomes and microvesicles are secreted by the progenitor cells in a normal adult mouse heart. They have also demonstrated a possible uptake of exosomes by cardiomyocytes in the adult mouse heart. However, questions still remain about the secretion of exosomes from the mammalian heart under ischemic conditions. We investigated exosome secretion from both human (healthy as well as chronic heart failure patients) and mouse (normal as well as post-MI) hearts. Electron micrographs of cardiomyocytes from the left ventricle of a healthy human as well as from a patient demonstrated the presence of double-membrane-bound, exosome-like vesicles of ≈50 nm size, enclosed within MVEs in the cytoplasmic area (Figure 2A and 2B). Our observations reveal for the first time that human cardiomyocytes can produce exosome-like vesicles via MVE-dependent pathway. Moreover, cardiomyocytes processed from the border zone of a mouse heart 6 hours after coronary artery occlusion display the presence of several cup-shaped exosomes of size ≈50–100 nm in the intracellular space embedded between the sarcomeres, nucleus, and a T-tubule, although not enclosed within a MVE-like structure (Figure 3A). We speculate that intact exosomes found inside the cardiomyocytes are either originated by an MVE-independent mechanism or uptaken directly from the extracellular space post-MI, without fusing to the plasma membrane. Direct internalization and active transportation of exosomes via endocytotic pathways to the perinuclear region is known to be mediated by the cytoskeleton and has been shown in neuronal-like PC12 cells.80 Barile et al80 discussed the uptake of intact exosomes by cardiomyocytes within small, cytoplasmic structures. We also detected double-membrane-bound, exosome-like vesicles packed in MVEs of a cardiac progenitor cell characterized by a large nucleus and a thin cytoplasm (Figure 3B) in a healthy mouse heart. Next, we examined whether exosomes secreted by the heart tissues can be experimentally separated from the cellular material and other types of secretory vesicles. As our dynamic light scattering analyses implies, we could isolate exosomes (ranging in size from 30 to 120 nm that float on 30% sucrose-D2O) successfully from both normal and ischemic heart, without any significant contamination from other types of vesicles or cell debris (Figure 3C). Collectively, this evidence suggests that both human and mouse cardiomyocytes secrete exosomes under healthy and ischemic conditions. It will be interesting to examine the distribution, fate, and physiological function of cardiac-derived exosomes and to investigate whether the secreted exosomes are targeted to function in particular cells/organs.

Cardiomyocytes Secrete Exosomes That Shuttle Proteins and Genetic Information to Other Cells: In Vitro Evidence

One of the limitations of in vivo studies is that there is no established method to distinguish exosomes secreted from a specific cell type, because the extracellular space has mixed exosomes from all cellular sources. Therefore, in vitro studies using a single cell type are useful to examine the content and function of exosomes from that cell type. Several in vitro reports using primary rodent cardiomyocytes have provided evidence of exosome secretion.53,81-83 In one of the earliest studies, Gupta and Knowlton81 demonstrated that highly differentiated adult cardiomyocytes, generally not considered a

![Figure 3. Presence of exosomes in a cardiomyocyte from the ischemic zone of a mouse heart (A), processed 6 h after myocardial infarction. E indicates exosomes; N, nucleus; and T, T-tubule. Exosomes within a multivesicular body (MVB) in the cytoplasmic space of a stem cell from the ischemic zone of a mouse heart (B). Dynamic light scattering analysis of extrapure exosomes (which floated on a 30% sucrose-D2O), indicating a homogenous population of vesicles from both normal (left) and ischemic (right) hearts (C). Tissues were collected from the left ventricle 6 h after induction of ischemia either for electron microscopy analysis or for exosomes isolation. For exosomes isolation, the tissue was minced, digested with 0.1% collagenase type 1 for 20 minutes at 37°C, passed through 40-micron strainer to remove big debris, and exosomes isolated as described before.47](image-url)
### Table. Exosomes and Circulating miRNAs After Myocardial Infarction

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<td>miR-1; miR-133a; miR-214</td>
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<td>Tumor exosomes transfer oncoprotein Met to reprogram the BM and mobilize BM progenitor cells</td>
<td>Human, mouse</td>
<td>Met oncoprotein</td>
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secretory cell type, release a cytosolic protein, HSP60, within exosomes. Exosome secretion from the cardiomyocytes was independent of necrosis and was significantly increased under hypoxic stress. Extracellular HSP60, when not in exosomes, causes cardiac myocyte apoptosis via the activation of Toll-like receptor 4; thus, the release of HSP60 from exosomes is damaging to the surrounding cardiomyocytes. A recent work from the same group demonstrated that pathological changes in the environment, including fever, changes in pH, hypoxia, and ethanol treatment, alter the rate of exosomes secretion and the protein content of exosomes from adult cardiomyocytes. Furthermore, the authors have shown using the mass spectrometry analysis of adult primary cardiomyocyte-derived exosomes that the protein content of cardiac exosomes differ significantly from other types of exosomes described in literature and contain cytosolic, sarcomeric, and mitochondrial proteins. Similar observations were made by 2 independent reports demonstrating the release of TNF-α and HSP60 via exosomes from cultured cardiomyocytes. Waldenström et al detected nucleic acid–containing microvesicles/exosomes in the media of cultured cardiomyocyte cell line HL-1, which could reprogram the fibroblast gene expression. These investigations depict a new concept in cardiomyocyte communication, proposing that exosomes generated by cardiomyocytes are able to transfer protein or genetic information to other cells. However, these data should be interpreted with caution, because the secretory potential of a cell line could be different compared with cardiomyocytes in vivo. Although the role of exosomes is best studied using cultured cardiomyocytes, it is not clear whether exosomes are secreted by the heart, it is not clear whether exosomes have any physiological function, either locally, for example, in heart remodeling and repair, or distally, for example, in BM reprogramming, to mobilize stem and progenitor cells in response to an ischemic insult. Beyond the question of whether exosomes are secreted by the heart, it is not clear whether exosomes have any physiological function, either locally, for example, in heart remodeling and repair, or distally, for example, in BM reprogramming, to mobilize stem and progenitor cells in response to an ischemic insult. Beyond the question of whether exosomes are secreted by the heart, it is not clear whether exosomes have any physiological function, either locally, for example, in heart remodeling and repair, or distally, for example, in BM reprogramming, to mobilize stem and progenitor cells in response to an ischemic insult. Beyond the question of whether exosomes are secreted by the heart, it is not clear whether exosomes have any physiological function, either locally, for example, in heart remodeling and repair, or distally, for example, in BM reprogramming, to mobilize stem and progenitor cells in response to an ischemic insult. Beyond the question of whether exosomes are secreted by the heart, it is not clear whether exosomes have any physiological function, either locally, for example, in heart remodeling and repair, or distally, for example, in BM reprogramming, to mobilize stem and progenitor cells in response to an ischemic insult.

**Table.**

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AB indicates apoptotic body; BM, bone marrow; BMC, blood mononuclear cell; CHF, congestive heart failure; CLI, critical limb ischemia; EOC, early outgrowth cells; IR, ischemia/reperfusion; LAD, left anterior descending artery; MI, myocardial infarction; miRNA, microRNA; and MNC, mononuclear cells.
miR-133a are increased in the serum of patients with acute coronary syndrome, and that these miRNA levels correlate with serum cardiac troponin T levels. Using a mouse model, the authors have shown that the origin of these miRNAs is the infarct region and the border zone, and that miR-133a is released in exosomes derived from cardiac H9C2 cells. The authors concluded that the circulating miRNAs released from the injured myocardium after MI can be carried to distant organs via exosomes. In support of this concept, Cheng et al. detected significantly higher levels of miR-1 and miR-208 in exosomes from the urine of acute MI patients and in the circulating blood of rats after acute MI. Furthermore, a cardioprotective miR-214 has been shown to be upregulated in the heart after ischemia and altered in the plasma from coronary artery disease patients, indicating the severity of the disease; miR-214 has been shown to be secreted via exosomes from human endothelial cells. These studies indicate that cardiac-derived intraexosomal miRNAs are stable and are protected from degradation by the RNase present in plasma, and these could also be released in the urine. Moreover, as these miRNAs are released independently of myocardial apoptosis, or even before the release of cardiac troponin T, these could function as biomarkers for the early detection of acute MI. Interestingly, several reports suggest circulating miR-126 to be an important miRNA to indicate the damage and repair mechanisms in acute MI patients. Zampetaki et al. detected miR-126 to be part of a miRNA signature associated with MI in general patient population. They inferred that the activation of proangiogenic endothelial miR-126 in the plasma after ischemia/reperfusion injury in patients can be an indication of vascular injury and increased cardiovascular risk. Our data and that of others suggest that circulating human CD34+ stem cell–derived exosomes are enriched with miR-126, and upregulation in the circulation can also imply the mobilization and release of exosomes by the stem cells. Interestingly, De Rosa et al. demonstrated a reduction in the expression and uptake of miR-126 in the transcoronary circulation of patients with coronary artery disease, indicating the consumption of proangiogenic miR-126 by the damaged heart. The same study reported an increase in miR-499 and 133a across the transcoronary circulation. Beside these roles, miR-126 is reported to induce the expression of CXCL12, promoting the recruitment of progenitor cells by a feedback mechanism. This earlier work also showed that in addition to exosomes other vesicles, such as apoptotic vesicles, released by the heart might be important for signaling to and from BM. Further experimental studies are necessary to explore the mechanism(s) by which MI and therapeutic interventions affect tissue versus circulating exosomal miRNA levels.

**Exosomal miRNAs Reprogramming the BM**

In a pioneering work, Peinado et al. provided persuasive evidence that in order to spread metastasis the tumor (melanoma) cells must release exosomes containing oncoprotein MET not only to the cancer microenvironment, but also to distant organs such as BM and lungs. The authors proposed a novel mechanism by which circulating exosomes from a tumor could cross-talk, reprogram, and permanently educate the BM progenitor cell to mobilize out of the BM. This process, they suggested, will contribute to a switch from a localized disease to disseminated, metastatic disease. Although the specifics in this work related to cancer biology are intriguing, we think that their findings have much broader implications, providing evidence of the complexity of the cargo carried by exosomes and its enormous potential to directly influence the biology of distant microenvironments. This important study has obvious potential implications in cardiology and should stimulate further research into the role of exosomes from the sick myocardium and its cellular targets in the BM. In a parallel study, Aliotta et al. demonstrated that the lung-derived microvesicles enter into the marrow cells in mice to deliver mRNA and induce the expression of lung-specific mRNA in the BM. The authors also demonstrated the tissue-specific expression of brain, heart, and liver mRNA in cocultured marrow cells in vitro. These experiments suggest that the exosome/microvesicle-mediated change in cellular phenotype is a universal phenomenon and, in theory, could be applicable to myocardium-derived exosomes reprogramming the BM environment.

Figure 4. A suggested hypothesis on the role of exosomes released from a damaged heart as a potential intercellular communicator. Exosomes can carry signaling molecules to activate local tissues (C indicates cardiomyocytes; E, endothelial cells; F, fibroblasts; and S, stem cells) and distant organs such as bone marrow (BM). Furthermore, the exosomes released from progenitor cells and the reprogrammed BM can reprogram the ischemic tissues of the heart, inducing protection and regeneration (illustration credit: Ben Smith).
In support of this notion, several recent reports demonstrate that acute MI modulates the miRNA expression of BM cells both in humans and in mice.3,9,10 Jakob et al9 reported that peripheral blood–derived CD34+ stem cells and early outgrowth cells have significantly reduced the levels of proangiogenic miRNA-126 and miR-130a in patients with chronic heart failure caused by ischemic cardiomyopathy as compared with healthy subjects. Other reports in mouse models suggest that cardiac ischemia mobilizes BM mononuclear cells via downregulating the expression of miR-150 activating CXCR4 in BM mononuclear cells.96 miR-34a, miR-126, miR-130a, and miR-150 are known to be present in serum-derived exosomes.95,97,98 Collectively, this evidence strongly supports the proposition that cardiac exosomes released after ischemic insults affect the BM microenvironment to reprogram the BM cells and initiate a repair process.

**Perspective**

Mounting evidence suggests that exosomes released from a damaged or diseased heart could be a potential intercellular communicator and carry signaling molecules to activate distant organs such as BM (Figure 4). However, the field of cardiovascular exosomes, which is currently in its infancy, needs to take significant steps to address several unanswered questions and challenges before arriving at this conclusion. Some of the important issues to explore are: (1) What type(s) of cells initiate signaling after ischemic insult in the myocardium? (2) Do different regions of the myocardium (eg, the infarct/border zone) release quantitatively and qualitatively different exosomes? (3) What is the fate of cardiac-derived exosomes in terms of uptake and potential downstream mechanisms? (4) Do cardiac-derived exosomes have direct effects on the reprogramming of local stem cells in the myocardium or remotely in the BM or both? Their infinitely small size, difficulty in studying them under physiological conditions, dynamic release and uptake of vesicles in tissues and fluids, and unknown efficiency of purification and quantification are some of the practical challenges in the study of exosomes. Nonetheless, the benefits of studying exosomes as an extracellular communicator in cardiac disease are multifold. We think that the study of exosomes will illustrate novel mechanisms of cell–cell and organ–organ communication, identify novel biomarkers of the disease, aid in our understanding of the mechanism of cell therapies for ischemia, provide insights for the development of novel therapeutics, and reveal the mechanisms of cell targeting (eg, hard–to-transfect cardiomyocyte targeting) for the discovery of novel candidates and the delivery of therapeutic compounds for cardiovascular diseases.

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**Disclosures**

None.

**References**


Exosomes in Myocardial Repair After Ischemia

Sahoo and Losordo


Exosomes and Cardiac Repair After Myocardial Infarction
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