Biochimica et Biophysica Acta 1852 (2015) 1–11

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbadis

Review

Pathologic function and therapeutic potential of exosomes in cardiovascular disease

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ARTICLE INFO

Article history:
Received 12 August 2014
Received in revised form 6 October 2014
Accepted 9 October 2014
Available online 16 October 2014

Keywords:
Exosomes
Cardiomyopathy
Cardiac remodeling
Stem cells
miRNAs

ABSTRACT

The heart is a very complex conglomeration of organized interactions between various different cell types that all aid in facilitating myocardial function through contractility, sufficient perfusion, and cell-to-cell reception. In order to make sure that all features of the heart work effectively, it is imperative to have a well-controlled communication system among the different types of cells. One of the most important ways that the heart regulates itself is by the use of extracellular vesicles, more specifically, exosomes. Exosomes are types of nano-vesicles, naturally released from living cells. They are believed to play a critical role in intercellular communication through the means of certain mechanisms including direct cell-to-cell contact, long-range signals as well as electrical and extracellular chemical molecules. Exosomes contain many unique features like surface proteins/receptors, lipids, mRNAs, microRNAs, transcription factors and other proteins. Recent studies indicate that the exosomal contents are highly regulated by various stress and disease conditions, in turn reflective of the parent cell status. At present, exosomes are well appreciated to be involved in the process of tumor and infection disease. However, the research on cardiac exosomes is just emerging. In this review, we summarize recent findings on the pathologic effects of exosomes on cardiac remodeling under stress and disease conditions, including cardiac hypertrophy, peripartum cardiomyopathy, diabetic cardiomyopathy and sepsis-induced cardiovascular dysfunction. In addition, the cardio-protective effects of stress-preconditioned exosomes and stem cell-derived exosomes are also summarized. Finally, we discuss how to epigenetically reprogram exosome contents in host cells which makes them beneficial for the heart.

1. Introduction

Cardiovascular disease is the number one cause of death in human beings, with approximately one in every four deaths being cardiac related [1]. The mammalian heart is thought of as a highly stressed organ which continually keeps beating and outputs blood/nutrition to the whole body. To maintain effective myocardial function, synchronized communication among different cell types within the heart is needed. It is estimated that the human heart contains two to three billion cardiomyocytes, which account for approximately one-third of the total number of cells in the heart [2]. This balance includes a wide array of additional cell types, such as smooth muscle cells, endothelial cells, fibroblasts, mast cells, immune system-related cells, other connective tissue cells and pluripotent cardiac stem cells [2–4]. It is becoming clear that a number of local and long-distance cell–cell communications contribute to the maintenance of normal cardiac homeostasis as well as responses to hypertrophic stimuli [4–12]. These include growth factor-mediated paracrine/autocrine and adiponectin-mediated endocrine signals, gap junction-mediated cell–cell contacts, and cell–matrix interactions and signaling through adhesion molecules including integrins that may modulate ventricular growth in crosstalk with circulating growth factors and other humoral mediators [4–12].

Recently, a group of small membrane vesicles naturally released from mammalian cells have been implicated as a new tool for cardiac cell communication [13–15]. These extracellular vesicles have a fluid lipid bilayer and contain a specific set of proteins, genetic materials, and lipids [13–15]. The types of membrane vesicles produced from cardiac cells include apoptotic bodies, microvesicles and exosomes [15]. These different vesicles are distinguished from one to another on the basis of their subcellular origin, size, and content. Apoptotic bodies (1–5 μm in diameter) are released from the plasma membrane of cells as blebs when they undergo apoptosis [14–16]. They contain a variety of cellular organelles, intracellular fragments, histones and even fragmented DNA and can be detected by extensive amounts of phosphatidylserine. Apoptotic bodies are considered to be mobile as they have the ability to float on a sucrose substance with a density between 1.16 and 1.28 g/mL [14–16]. While the exact role of apoptotic bodies is not fully understood, it is believed that apoptotic bodies could be used in activating cell signals to promote the elimination of

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http://dx.doi.org/10.1016/j.bbadis.2014.10.008
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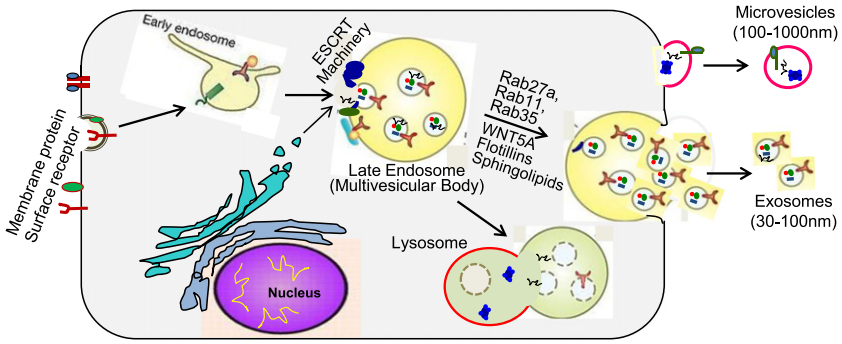

Fig. 1. The generation of microvesicles and exosomes. Microvesicles (MVs) are formed directly by outward budding or blebbing of the plasma membrane. Specific loading of membrane proteins, lipids and RNAs to the MVs is known to occur, but the exact molecular mechanism are largely unknown. Biogenesis of exosomes is initiated with inward budding of the cell membrane, with specific membrane proteins/receptors incorporated, to form early endosomes. Subsequently, the cargo is packaged into intraluminal vesicles (ILVs) upon second inward budding of the early endosome membrane, and then transformed into multivesicle bodies (MVBs). Four different mechanisms have been described to facilitate the cargo loading: 1) ESCRT machinery and associated proteins; 2) lipid rafts; 3) higher-ordered oligomerization; and 4) segregation into microdomains by ceramide. MVBs can then fuse with the lysosomal membrane and release ILVs for degradation. Alternatively, MVBs fuse with the plasma membrane and release ILVs into the extracellular space as exosomes, a process which is regulated by Rab27a, Rab11, Rab35, WNT5A, SNAREs, glycosphingolipids and flotillins, etc.

other damaged cells [15]. Usually, microvesicles (100–1000 μm) are generated from cells by a direct outward budding of the cell membrane when they are exposed to certain stimuli including exposure to prothrombotic and pro-inflammatory as well as cellular differentiation and senescence [14–16]. Unlike apoptotic bodies, microvesicles float on a different sucrose gradient of 1.04–1.06 g/mL and are usually derived from platelets, endothelial and red blood cells [15]. Microvesicles contain a variety of bioactive molecules that include a variety of adhesive structures, cytokines, chemokines, cytoplasmic proteins, membrane proteins, cellular receptors, non-coding RNA, and even mRNA and miRNAs. The various different protein markers for microvesicles include integrins, selectins, and the CD40 ligand [15]. Exosomes are types of extracellular vesicles whose sizes range between 30–100 nm and float to a density ranging from 1.13–1.19 g/mL using ultracentrifugation on linear sucrose gradient (2 M–0.25 M sucrose) [15–18]. They have very unique qualities that greatly set them apart from other extracellular vesicles (apoptotic bodies and microvesicles). Secretion of exosomes from viable cells was proposed to be a mechanism through which cells discard their cellular waste [19–22]. However, over the past years, exosomes have emerged as important players for inter-cellular communication that are involved both in normal physiology, e.g. cardiac development, myocardial angiogenesis, and vesicle formation during reticulocyte maturation [13,15,20–22], and in pathophysiological conditions, e.g. progression of cancer, liver disease, immune-defective disease, and neurodegenerative disease [16,23–25]. In this review, we will focus on exosomes and their role in cardiac patho-physiology. Specifically, we will: 1) discuss how to regulate exosome biogenesis and secretion, 2) summarize the advances on the detrimental and beneficial role of exosomes in cardiac remodeling, and 3) discuss the therapeutic potential of exosomes in cardiovascular diseases.

2. Biogenesis and secretion of exosomes

2.1. Exosome biogenesis

The term “exosomes” was coined by Johnstone et al. [21] in their investigation of vesicle formation during reticulocyte maturation. Since their initial discovery, much has been learned about the biogenesis of exosomes. Unlike microvesicles that are derived from the direct outward blebs of plasma, exosomes are generated within the endosomal network, beginning with the inward budding of the cell membrane to form early endosomes, followed by second inward budding of the endosomal membrane to form the various intraluminal vesicles (ILVs) (late endosomes). The late endosomes containing ILVs are also called multivesicular bodies (MVBs). Finally, the MVBs may fuse with the cell membrane, releasing its ILVs in an exocytotic fashion to the extracellular environment. These ILVs are then referred to as exosomes. Alternatively, the MVBs move towards the perinuclear area of the cell where they can directly fuse with lysosomes, creating hybrid organelles where endocytosed cargos are degraded. The process of exosome biogenesis is summarized in Fig. 1.

Nowadays, the molecular mechanisms of ILV (exosome) formation and sorting of endosomal proteins into these vesicles are partially understood. The best described machinery that drives the process of MVB formation is coordinated by the endosomal sorting complexes required for transport (ESCRT) system, which has been widely reviewed recently in the literature [26–29]. In brief, this machinery consists of four soluble multi-protein complexes, called ESCRT-0, ESCRT-I, ESCRT-II and ESCRT-III, and its associated proteins. ESCRT-0 is responsible for cargo clustering in an ubiquitin-dependent manner; ESCRT-I and ESCRT-II induce bud formation; ESCRT-III drives vesicle scission, and the accessory proteins (especially the VPS4 ATPase) allow dissociation and recycling of the ESCRT machinery. Recently, Colombo et al. [30] performed an RNA interference screen targeting 23 components of ESCRT complex and associated proteins in HeLa cells. They observed that depletion of HRS, STAM1 (ESCRT-0 members), and Tsg101 (ESCRT-I component) inhibited exosome biogenesis; by contrast, silencing of VPS4b (ESCRT-III member) promoted exosome formation. Notably, Colombo et al. [30] observed that members of ESCRT-II did not have any influences on exosome biogenesis/release in HeLa cells. Of interest, several recent studies show that the ESCRT-III-associated protein, ALIX, can augment intraluminal budding of vesicles in endosomes and enhance CD63-positive exosome biogenesis in primary dendritic cells (DCs) and muscle cell line C2C12 [31–33].

Although many studies use ESCRT silencing as a tool to inhibit biogenesis of exosomes, one should be aware that ESCRT-independent mechanisms are also involved in the ILV formation and exosome biogenesis [34,35]. These mechanisms are associated with proteolipid proteins, tetraspanins or heat shock proteins (see reviews elsewhere [26]). Currently, it remains obscure whether these multiple mechanisms of biogenesis of exosomes/ILVs can occur in a single MVB, or different MVB populations can co-exist within the cell. In addition, it is not completely known how to decide different MVB subpopulations' fate for either lysosomal degradation or exosomal release. Future studies will be needed to clarify such issues.

2.2. Exosome secretion

Exosomes can be released from the cell by two mechanisms: constitutive or inducible. The constitutive secretion pathway is regulated by certain RAB GTPases (Rab27a/b, Rab11 and Rab35), heterotrimetric G-protein, WNT5A, glycosphingolipids and flotillins [36–41]. On the

other hand, the inducible secretion is regulated by stress stimuli including aberrant intracellular calcium release, DNA damage, thrombin, heat shock, hypoxia and lipopolysaccharide (LPS) stimulation [42–51]. For example, we and others have shown that a low dose of H_2O_2 (20 μM) can stimulate cardiomyocytes to produce exosomes [48–50]. Recent studies have also shown that ischemic preconditioning can induce exosome secretion from the heart [52]. In addition, change in membrane pH has been shown to trigger the secretion of exosomes from various cell types [49,53].

Regardless of whether it is a constitutive or inducible release, the final step of exosome secretion requires the fusion of MVBs with the plasma membrane. This process possibly involves a specific combination of Soluble N-ethylmaleimide-sensitive factor-attachment protein receptors (SNAREs): vesicular SNAREs (v-SNAREs, localized on MVBs) interact with target SNAREs (t-SNAREs, localized on the intracellular side of the plasma membrane), to form a membrane-bridging SNARE complex, responsible for membrane fusion [54,55].

2.3. Molecular composition of exosomes

Increasing evidence shows that exosome composition contains both a common and a specific set of DNA, RNA and proteins (see database available at www.exocarta.org; www.evpedia.info) [56,57]. The common set of exosomal proteins that have been identified are found in parent's cell membranes of endocytic compartment or the plasma membrane, also in the cytosol, the Golgi, even the nucleus, but rarely the endoplasmic reticulum or mitochondria [56–58]. The typical membrane proteins encased in exosomes include GPI-anchored proteins, Tetraspanins (CD81, CD82 CD63, and CD9) and receptors [i.e., Tumor Necrosis Factor Receptor 1 (TNFR1)]. Other typical types of molecules wrapped in exosomes include: 1) luminal proteins such as annexin 2 and cytokines; 2) antigen-presentation (MHC-I, MHC-II) and co-presentation molecules (CD86), cell adhesion (integrins, MFGE8, etc.), cell structure and motility (actins, myosin, tubulin, etc.); 3) heat shock proteins and chaperones (Hsp90, Hsp70, Hsp60, Hsp20, αB-Crystalline); 4) metabolic enzymes (β-enolase, fatty acid synthase, glyceraldehyde-3-phosphate dehydrogenase, peroxidases, pyruvate kinase); 5) proteins involved in exosome biogenesis (ESCRT complex, i.e. Tsg101, Alix); 6) signaling proteins (kinases, 14-3-3, GTPase Hras, RhoA, RAP1B, guanine nucleotide-binding protein subunits—G proteins, etc.); 7) proteins involved in transcription and protein synthesis (transcription factors, histones, ribosomal proteins, ubiquitin, etc.), and 8) proteins involved in trafficking and membrane fusion (Annexins, Rab protein family, ARF).

In addition to a common set of proteins, it has been characterized that exosomes display a particular lipid organization and composition (see reviews elsewhere [18,59,60]). A recent unexpected finding showed that exosomes contain double-stranded DNA, mRNA and non-coding RNA (microRNA and lncRNA) [61–64]. Importantly, we and others have demonstrated that these exosomal mRNA and microRNA are functional in recipient cells [65–69]. This suggests that the exchange of exosome contents between cells may represent an effective and efficient inter-cellular communication. Furthermore, it is now known that protein and RNA sorting into exosomes is highly regulated by various patho-physiological stress stimuli and disease conditions [46,64]. This allows cells to produce tailor-made exosomes with different functional characteristics, reflective of their parent cell status. In this regard, any stress or disease conditions may be mirrored in the contents of the exosomes, which can be used to develop future biomarkers for the diagnosis and prognosis of diverse cardiovascular disease.

3. Pathological effects of exosomes on cardiac remodeling

At present, exosomes have been implicated to mediate cell-to-cell communication through different mechanisms of interaction with recipient cells, including: 1) direct ligand–receptor interaction, resulting in activation of downstream signaling, 2) extracellular proteases cleave exosomal membrane proteins, releasing soluble ligands that bind to target receptors of recipient cells, 3) direct membrane fusion, leading to release of exosomal contents into recipient cells, and 4) internalization of exosomes by endocytic mechanisms (phagocytosis, macropinocytosis or receptor-mediated endocytosis) [70]. Considering that the multiple properties of exosomes (reviewed above) and the secretion of exosomes from a variety of different cell types within the heart, it is reasonable to expect that exosomes are likely to be involved in many cardiovascular physiological and pathological disorders. However, the published studies on such issues are scarce. This may be ascribed to either a lack of knowledge or some technical difficulties on exosomes. As a matter of fact, we only collect and summarize a small number of recent publications that investigated the roles of exosomes in cardiomyocyte hypertrophy, peripartum cardiomyopathy, diabetes-, and sepsis-induced cardiomyopathy, as reviewed below.

3.1. Exosomes in cardiomyocyte hypertrophy

It is now known that cardiac stress (i.e. systemic hypertension, cardiac valve disease, and myocardial infarction) can lead to cardiomyocyte hypertrophy, fibroblast proliferation, and secretion of extracellular matrix proteins and proinflammatory cytokines [71–73]. These fibroblast-derived mediators may act in either an autocrine or paracrine fashion between fibroblasts and cardiomyocytes to further stimulate cardiac remodeling [73,74]. Notably, cardiomyocytes comprise only 56% (mouse), 30% (rat), and 28% (human) of all the cells in the healthy adult heart; whereas cardiac fibroblasts have been reported to make up 27% (mouse), 64% (rat), and 70% (human) of total cardiac cells [75]. Traditionally, cardiac fibroblasts are considered to play passive roles in the heart and to be solely responsible for maintaining homeostasis of extracellular matrix proteins, including type I and III collagens and fibronectin [76]. However, when co-cultured with cardiac fibroblasts or treated with conditioned fibroblast media, adult murine cardiomyocytes develop hypertrophy [77,78]. This suggests that cardiac fibroblast may be active participants in cardiomyocyte remodeling, but the underlying mechanisms of fibroblast-cardiomyocyte communication are not yet fully understood.

Recently, Bang and co-workers [68] reported a unique mechanism in which cardiac fibroblasts promote the hypertrophic response of cardiomyocytes through exosomal transfer of miR-21* (Fig. 2). They firstly identified that cardiac fibroblast produce and secret exosomes. Using a RNA sequencing approach, these authors analyzed that 388 rat miRNAs and 50 miRNAs were found to be enriched in exosomes derived from neonatal rat cardiac fibroblasts. Interestingly, they observed that 25.5% of fibroblast exosomal miRNAs were so called “star” miRNAs, the passenger (star) strand that is often degraded within a cell. In particular, the levels of miR-21* were significantly higher in exosomes, whereas miR-21 expression was higher in their donor fibroblasts, when compared to each other. This data indicates that miR-21* is selectively packaged into exosomes. Nonetheless, it is too early to conclude that miRNAs remain inside of donor cells, whereas miRNAs* may be kicked out of cells through exosomes. The most important finding by Bang and colleagues [68] is that, fibroblast-derived exosomal miR-21* can be taken up by cardiomyocytes, which is dependent on temperature and actin. Of significance, such exosomal miR-21*, once released within cardiomyocytes, is functional and down-regulates the expression of SORBS2 (sarcoplasmic protein sorbin and SH3 domain-containing protein 2) and PDLIM5 (PDZ and LIM domain 5), leading to a substantial increase in cardiomyocyte cell size. Moreover, the study by Bang et al. [68] may support previous findings showing that miR-21* was detected in pericardial fluid of mice with transverse aortic constriction-induced cardiac hypertrophy [79], thus confirming in-vivo that miR-21* plays a critical role in regulation of the cardiac fibroblast secretome and in determining a hypertrophic response.

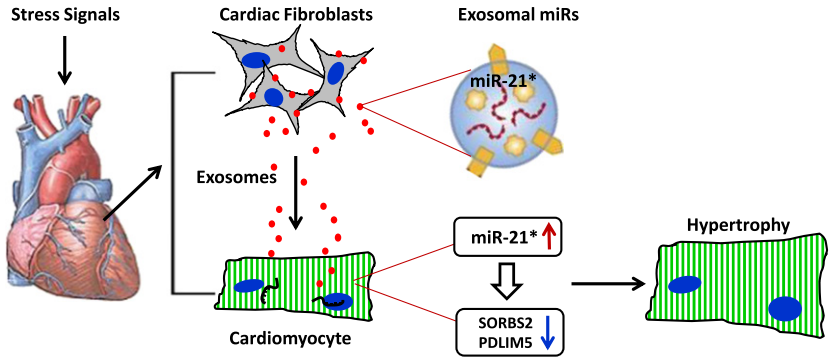

Fig. 2. Under stress conditions, cardiac fibroblasts secret miR-21*-enriched exosomes, which are taken up by cardiomyocytes, leading to elevation of miR-21*. Consequently, the expression levels of SORBS2 and PDLIM5 are down-regulated in cardiomyocytes, resulting in cardiomyocyte hypertrophy.

Overall, under stress conditions, cardiac fibroblasts can promote an undesirable pathologic hypertrophy of cardiomyocytes through exosomes and their miRNA cargo. However, many questions remain unclear. For example, the development of fibroblast during cardiomyopathy involves multiple types of pro-fibrotic cells which may be derived from epithelial–mesenchymal transition (EMT), endothelial–mesenchymal transition (EndMT), perivascular cells, circulating monocytes/fibrocytes, or bone marrow originated progenitor cells. Therefore, it will need to clarify whether and how exosomes contribute to such a process of cardiac fibrosis. Given that cardiac fibroblast not only insulate myocyte bundles but also integrate to myocytes through connexin proteins, it will be an urgent need to address whether cardiac exosomes play a role in cardiac electrophysiology. In addition, it will need to investigate whether exosomes are involved in both synthesis and degradation of extracellular matrix to form fibrosis in the heart.

3.2. Exosomes in peripartum cardiomyopathy

Peripartum cardiomyopathy (PPCM) is a very dangerous pregnancy-associated cardiomyopathy that resides in a large population of women [80,81]. It is characterized by sudden heart failure during the last month of pregnancy and/or in the first few months of postpartum. A 16-kDa N-terminal prolactin fragment (16K PRL), cleaved from the full-length nursing hormone prolactin (PRL) by cathepsin D, is believed to be a potential factor in initiating PPCM [82]. The underlying molecular mechanisms, however, remain obscure. Recently, Halkein et al. [69] reported that 16K PRL not only induced the expression of miR-146a in endothelial cells (ECs), leading to inhibition of angiogenesis, but also enhanced the release of miR-146a-enriched exosomes from ECs (Fig. 3). These endothelial exosomes can be taken up by cardiomyocytes, resulting in the elevation of miR-146a levels. Consequently, the expression of Erbb4, Notch1, and Irak1 (targets of miR-146a) was decreased in cardiomyocytes, leading to impaired metabolic activity and contractile function. These findings provide evidence that shows a miRNA-based intercellular communication system between ECs and cardiomyocytes via exosomes. Moreover, in situ hybridization in postpartum STAT3-knockout mouse hearts mainly detected miR-146a in ECs and other nonmyocyte cardiac cells, such as cardiac fibroblasts. This suggests that in addition to ECs, fibroblasts may also be a source of exosomal miR-146a in hearts exposed to 16K PRL.

Of interest, Halkein et al. [69] further observed that levels of exosomal miR-146a were significantly higher in plasma from patients with acute PPCM ($n = 38$) than healthy postpartum controls ($n = 18$) and patients with dilated cardiomyopathy ($n = 30$). It is important to note here that 12 PPCM patients who recovered after being treated with standard therapy for heart failure (beta blocker and ACE inhibitor) and 2.5–5.0 mg/d bromocriptine for 6–8 weeks, had the levels of circulating exosomal miR-146a restored to normal range. Thus, exosomal

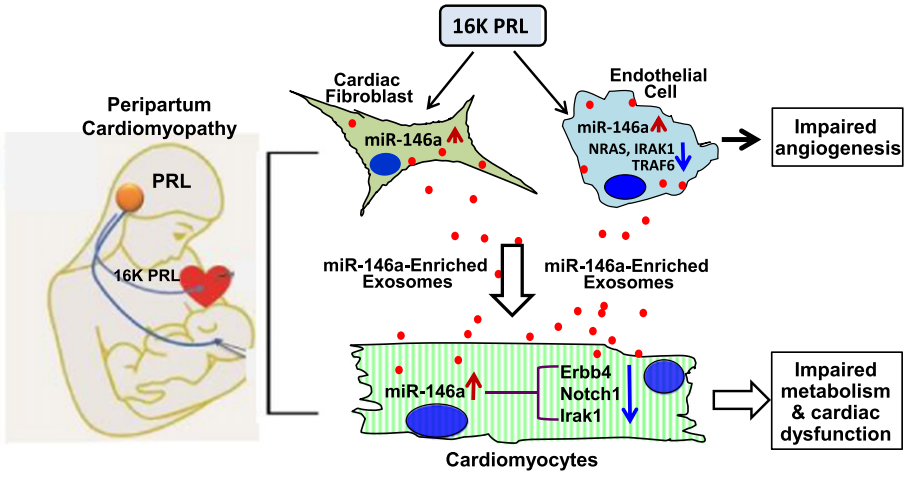

Fig. 3. In peripartum cardiomyopathy (PPCM) patients, cathepsin D cleaves nursing hormone prolactin (PRL) to generate an antiangiogenic 16-kDa fragment, 16K PRL. 16K PRL stimulates both cardiac fibroblasts and endothelial cells to release miR-146a-enriched exosomes, which transport miR-146a to cardiomyocytes. The exosome-mediated elevation of miR-146a in cardiomyocytes can down-regulate the expression of Erbb4, Notch1, and Irak1, leading to slowed metabolism and impaired contractile function in cardiomyocytes. In addition, 16K PRL stimulates endothelial cells to activate NF-κB, which up-regulates miR-146a expression, leading to decreased levels of NRAS, IRAK1 and TRAF6 and consequently, inhibiting angiogenesis.

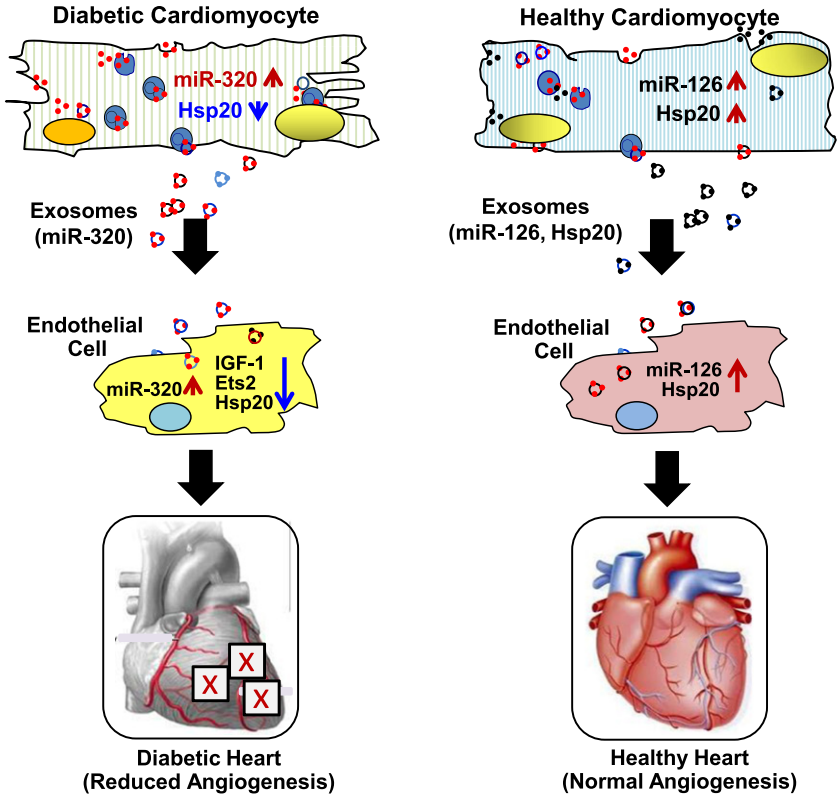

Fig. 4. In type-2 diabetic rat hearts, miR-320 is up-regulated, whereas Hsp20 is down-regulated in cardiomyocytes. Accordingly, exosomes released from diabetic cardiomyocytes contain higher levels of miR-320, compared with those from healthy cardiomyocytes. The exosomal miR-320 is then transported to endothelial cells, resulting in decreased levels of IGF-1, Ets2 and Hsp20 and thereby, inhibiting angiogenesis in diabetic hearts. By contrast, healthy cardiomyocytes can secret miR-126- and Hsp20-enriched exosomes, which transfer miR-126 and Hsp20 to endothelial cells, leading to angiogenesis.

miR-146a may serve as a highly specific blood biomarker useful for diagnosis and risk stratification of patients with peripartum heart failure.

3.3. Exosomes in diabetic cardiomyopathy

Diabetes affects approximately 8% of the United States population and it is predicted that, over 400 million people by 2030 will have cases of diabetes [83]. Diabetes mellitus is a group of metabolic diseases characterized by high blood glucose either due to lack of (type 1) or resistance to (type 2) insulin [84]. Early in the course of diabetes, high glucose levels in the bloodstream can lead to endothelial dysfunction and microvascular rarefaction [85,86]. Interestingly, insufficient myocardial angiogenesis is the major manifestation of diabetes-caused ischemic cardiovascular disease [87]. Within the mammalian heart, it is well recognized that endothelial cells play a critical role in cardiomyocyte survival and myocardial contraction [88]. However, in response to stress conditions (i.e. hyperglycemia), whether cardiomyocytes have an ability to affect endothelial cell function remains largely unknown. Recently, we and others have observed that exosomes derived from cardiomyocytes harbor a variety of mRNAs, miRNAs and proteins, which may be transferred to the adjacent endothelial cells and modulate their function [48,49,67,89]. In particular, we showed that when cardiac endothelial cells (ECs) were co-cultured with cardiomyocytes derived from type 2 diabetic Goto-Kakizaki (GK) rats, EC proliferation and migration were significantly inhibited, compared with EC culture alone [67]. By contrast, co-culture of ECs with cardiomyocytes isolated from healthy rats promoted EC proliferation and migration [67]. Thus, these data provide evidence that normal conditions of cardiomyocytes may benefit ECs, whereas diabetic myocytes may impair EC function (Fig. 4). Of interest, either beneficial or detrimental effects of cardiomyocytes on ECs were eliminated when treated with exosome inhibitor GW4869. Our further investigations revealed that exosomes derived from diabetic GK cardiomyocytes contain higher levels of miR-320, and lower levels of miR-126 and Hsp20 proteins, compared with exosomes collected from healthy cardiomyocytes [67]. Importantly, the cardiomyocyte exosomal miR-320 can be transferred to endothelial cells and consequently, down-regulates the expression of IGF-1, Hsp20, and Ets-2, leading to inhibition of EC proliferation, migration and tube formation [67]. Therefore, our study may provide a novel mechanism underlying the impairment of myocardial angiogenesis in diabetes which may be caused by secretion of anti-angiogenic exosomes from cardiomyocytes (Fig. 4).

3.4. Exosomes in septic cardiomyopathy

Cardiovascular dysfunction is a major player contributing to mortality associated with sepsis, a disease initiated by local severe infection [90]. There is compelling evidence that myocardial depression is present in >40% of sepsis patients [91], and that exosomes may be involved [92]. Sepsis is triggered by a reactive immune response to an infection that can potentially result in mass deterioration of multiple organs [90]. Inflammation induced by sepsis results in blood clotting that restricts blood flow, thus causing a lack of supply of nutrients and oxygen to vital organs, like the heart. As a matter of fact, cardiovascular dysfunction is a major factor contributing to mortality associated with sepsis [91]. Numerous studies have shown that biventricular dilation and decrease in ejection fraction are associated with septic patients [91]. Much of prior work has implicated cytokines (IL-6 and TNFα) and reactive oxygen/nitrogen species (nitric oxide, superoxide, and peroxynitrite) as myocardial depressors [90]. However, several recent studies indicate that the presence of exosomes in the plasma of sepsis patients may cause vascular and cardiac dysfunctions as well as coagulation in sepsis [51,92,93]. In particular, Gambim et al. [51] reported that in sepsis, both increased generation of nitric oxide (NO) and the

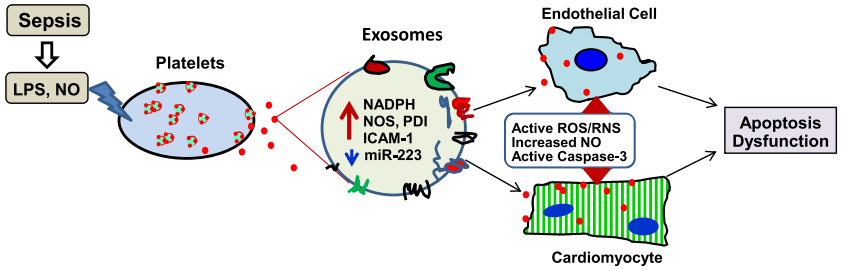

Fig. 5. Sepsis can cause higher levels of lipopolysaccharide (LPS) and nitric oxide (NO) in the blood, which stimulate platelets to secret inflammatory exosomes. Such exosomes are enriched with NADPH, NOS, PGI, ICAM-1 and less miR-223, which activate ROS/RNS signaling pathways, increase NO production and activate caspase-3 in both endothelial cells and cardiomyocytes, resulting in cell apoptosis and dysfunction and consequently, cardiomyopathy.

presence of LPS can trigger the release of exosomes from platelets. These septic exosomes contain higher levels of NADPH oxidase, nitric oxide synthases (NOS) and protein disulfide isomerase (PDI) than healthy exosomes, as evidenced by Western-blotting. Importantly, these authors observed that NO-induced and human septic platelet-derived exosomes induced caspase-3 activation and apoptosis of target endothelial cells through generation of active ROS/RNS by NADPH oxidase and NO synthase type II (Fig. 5). Azevedo et al. [92] further provided evidence that circulating platelet-derived exosomes from septic patients induced a decrease in myocardial contractility in isolated rabbit heart and rat papillary muscle preparations. These detrimental effects of exosomes were enhanced by pre-exposure to LPS. They confirmed that septic platelet-derived exosomes contain NOS, which can generate NO in exosomes and induce NO production in the heart. More recently, numerous studies have profiled circulating exosomal miRNAs in septic patients [63]. Specifically, the levels of exosomal miR-223 are significantly lower in the blood of non-survival septic patients, compared with survivors [94]. Of interest, reduced miR-223 may increase the expression of miR-223 targets (e.g. ICAM-1, IL-6, and IL-1β) [95–97]. Consequently, these increased target proteins may be enriched in septic exosomes and contribute to myocardial depression (Fig. 5). Put together, sepsis-induced cardiomyopathy may be mostly ascribed to both an increased number of exosomes and an intrinsic property of exosome (e.g. enriched NOS, reduced miR-223).

4. Protective effects of exosomes in the myocardium

As reviewed above, exosomes derived from disease conditions elicit detrimental effects on cardiac remodeling. Recent studies have also demonstrated that exosomes collected from ischemic preconditioning or various stem cells provide cardioprotection, which may have therapeutic potential in the treatment of cardiovascular diseases. In the following sections, we will summarize recent findings on the role of stem cell-derived, stress-preconditioned and gene-modified cell-derived exosomes in cardiac protection including enhanced myocardial angiogenesis, reduced oxidative stress, limited inflammatory response, decreased cardiomyocyte death and myocardial infarction size (Fig. 6).

4.1. Mesenchymal stem cell-derived exosomes confer protection in cardiovascular diseases

Various kinds of stem cells (embryonic stem cells, adult stem cells, and induced pluripotent stem cells) have been extensively investigated for the therapeutic potential in cardiovascular disease [98–100]. It was previously thought that stem cell therapy acts to regenerate tissue through replication and then differentiation, but recent studies indicate that the beneficial effects of adult stem cells in the repair of cardiac tissue is through the release of paracrine and autocrine factors [101]. Indeed, stem cells can secret numerous types of factors/molecules, including proteins, microRNAs, growth factors, antioxidants, proteasomes, microvesicles and exosomes. In particular, exosomes has garnered a specific attention in cell-free-based stem cell therapy for cardiovascular disease.

Lai et al. [102] first showed that human ESC-derived mesenchymal stem cells (MSCs) secreted 50- to 100-nm membrane vesicles. Using an ex-vivo Langendorff model of ischemia/reperfusion injury, they observed that these purified exosomes were able to reduce infarct size in mouse hearts. Arslan et al. [103] further demonstrated that a single intravenous bolus of exosomes 5 min prior to reperfusion reduced infarct size by 45% in mice. Importantly, they found that exosome treatment restored energy depletion and redox state in mouse hearts within 30 min after I/R, evidenced by elevation of ATP and NADH levels, and reduction of oxidative stress. This can be interpreted that MSC-derived exosomes contain all five enzymes [namely glyceraldehyde 3-phosphate dehydrogenase (GAPDH), phosphoglycerate kinase (PGK), phosphoglucomutase (PGM), enolase (ENO) and pyruvate kinase m2 isoform (PKm2), see database available at www.exocarta.org] required for ATP generation. In addition, active CD73 (the major enzyme responsible for the formation of extracellular adenosine from released adenine nucleotides) and the phosphorylated PFKFB3 (which upregulates phosphofructose kinase) are also wrapped in MSC-derived exosomes [103]. Moreover, exosome treatment could reduce systemic inflammation in mice after myocardial I/R [103]. Therefore, MSC-derived exosomes may have powerful therapeutic potential for patients suffering from acute myocardial infarction.

Similarly, Lee et al. [104] reported that the anti-inflammatory activity in mouse bone marrow-derived mesenchymal stem cells (MSCs) is associated with exosomes. Using a murine model of hypoxia-induced pulmonary hypertension (HPH), these authors demonstrated that MCS-derived exosome (MEX) delivery in-vivo suppressed HPH and vascular remodeling. Moreover, hyper-proliferative signals were also blocked by MEX treatment, as evidenced by the suppression of signal transducer and activator of transcription-3 (STAT3) phosphorylation, resulting in increased lung levels of miR-204, a microRNA enriched in distal pulmonary arterioles that is downregulated in both human PH and experimental models of disease. The data provided by Lee et al. [104] point to exosomes as the key effectors of MSC paracrine function. However, their work did not identify the critical components encased in these exosomes.

4.2. Hematopoietic stem cell-derived exosomes promotes myocardial angiogenesis

It is previously hypothesized that hematopoietic stem cells (HSCs) could repair cardiac tissue by differentiating into cardiomyocytes [105]. However, numerous studies have shown that few of the transplanted HSC survive and even fewer of the cells become cardiomyocytes [106]. Nonetheless, it has been observed that the transplantation of these HSCs improved cardiac function in both animal models and human patients [107–110]. This may be the result of a variety of paracrine factors including vascular endothelial growth factor (VEGF), FGF, HGF, and IGF, released from implanted HSCs. Recently, a study by Sahoo et al. [111] showed that human $CD34^+$ stem cells had

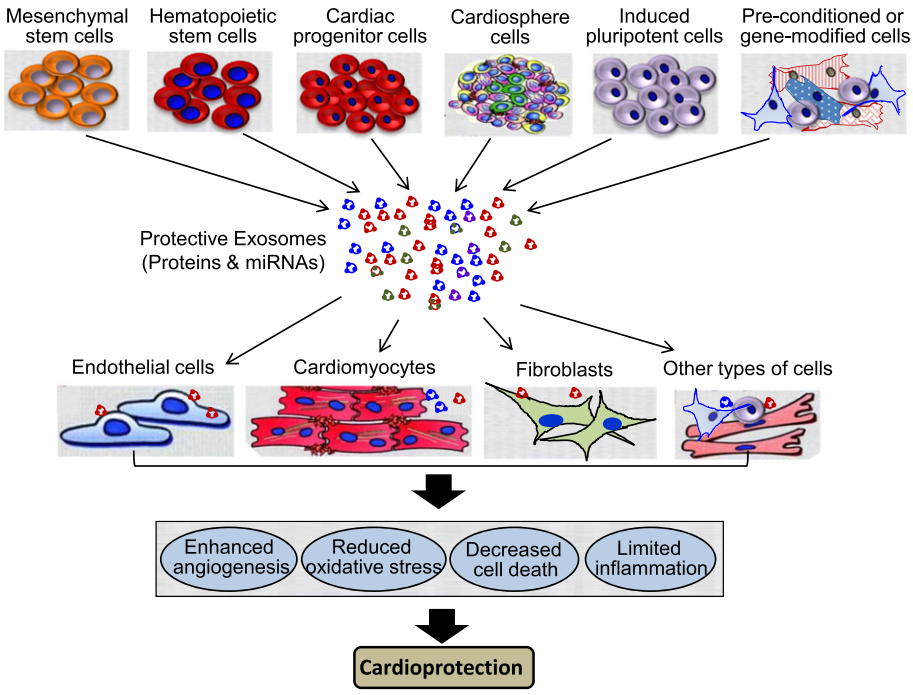

Fig. 6. Multiple sources of stem cells, stress-preconditioned cells and gene-modified cells can yield cardio-protective exosomes. These protective exosomes, when injected into hearts, can interact with endothelial cells, cardiomyocytes, fibroblasts and other types of cells within hearts by endocytosis, receptor/ligand-mediated action, or membrane fusion, resulting in enhanced angiogenesis, reduced oxidative stress, decreased cell apoptosis/necrosis, and limited inflammatory response.

the ability to secrete cup-shaped exosomes which expressed CD63, phosphatidylserine and TSG101. By the in vitro method, it was found that $CD34^+$-exosomes replicated the angiogenic activity of the $CD34^+$ cells by enhancing endothelial cell viability, proliferation and tube-like formation on Matrigel. In the in vivo studies, these authors observed that both $CD34^+$-cells and $CD34^+$-exosomes induced the formation of vessel-like endothelial structures, accompanied by a significantly increased proportion of endothelial cells in the Matrigel plug. In the corneal angiogenesis assay, pellets containing $CD34^+$-exosomes, but not $CD34^-$-exosomes, were associated with significantly greater vessel growth. Hence, these findings indicate that the $CD34^+$-exosomes are the key paracrine component of $CD34^+$-cell-induced vessel growth. Nonetheless, the mechanisms underlying $CD34^+$-exosome-mediated angiogenesis are not clearly understood. It could be ascribed to exosomal receptor-induced activation of angiogenic signaling cascades or the overall transfer of exosomal contents (proteins/RNAs) into the cytosol of endothelial cells. Indeed, Sahoo et al. [111] has presented data demonstrating that $CD34^+$-exosomes are highly enriched with pro-angiogenic miR-126 and miR-130a, compared with $CD34^-$-exosomes. However, the extent to which these exosomal miRs are transferred and induce any molecular changes in the recipient cells remains to be clarified.

While the benefit of $CD34^+$-cell therapy on functional recovery after ischemic injury could be induced primarily through the exosome-mediated transfer of angiogenic factors to surrounding cells, both viability and angiogenic quality of autologous $CD34^+$-cells decline with advanced age [112,113]. In this regard, Mackie et al. [114] recently attempted to genetically modify $CD34^+$-cells with the sonic hedgehog (Shh) gene ($CD34^{Shh}$) for enhancing their angiogenic quality. They observed that treatment with $CD34^{Shh}$ induced robust increases in capillary development within the infarct border zone, accompanied by reduced infarct sizes. Importantly, they found that $CD34^{Shh}$ deposits a greater amount of Shh in exosomes as compared with other Shh-modified cell types. Shh-containing exosomes derived from $CD34^{Shh}$ are capable of transferring Shh to other cell types (endothelial cells and fibroblasts) and thereby, activating the Shh signaling pathway. Thus, exosome-mediated delivery of Shh to ischemic myocardium may represent a major mechanism explaining the observed preservation of cardiac function in mice treated with $CD34^{Shh}$ cells.

4.3. Cardiac progenitor cells-derived exosomes provide cardioprotection

Cardiac progenitor cells (CPCs) can also release exosomes into their environment and those exosomes contain matrix metalloproteinases (MMPs) and extracellular matrix metalloproteinase inducer (EMMPRIN) that all aid in the breakdown of the extracellular matrix as well as activate MMP [115]. In addition, GTAT4-responsive miR-451 is highly enriched in these exosomes [116,117]. As a matter of fact, Vrijsen et al. [115] observed that CPC-derived exosomes can stimulate the migration of endothelial cells in an in vitro scratch assay. Chen et al. [116] found that in vivo delivery of CPC-exosomes in an acute mouse myocardial ischemia/reperfusion model inhibited cardiomyocyte apoptosis by about 53% in comparison with PBS control. Together, these studies implicate exosomes as the important cardioprotective component in CPC-mediated paracrine effects.

Currently, it is recognized that cardiac progenitor cells can be clonally expanded from murine and human myocardial biopsy specimens and form “spheres” in vitro which is referred to as cardiospheres (CSs) [118–121]. Because CSs contain both primitive cells and committed progenitors for the three major cell types present in the heart (cardiomyocytes, endothelial cells, and smooth muscle cells), they represent an attractive cell source for cardiac regeneration. Indeed, numerous studies have demonstrated that cardiosphere-derived cells (CDCs) can stimulate regeneration, angiogenesis, and functional improvement in the infarcted human heart [122–124]. Most recently, Ibrahim et al. [125] showed that CDC-derived exosomes can replicate CDC-induced therapeutic effects, and blockade of exosome production negates the beneficial effects of CDCs on the infarcted mouse hearts. They further identified miR-146a as being particularly enriched in CDC exosomes. Of interest, miR-146a may largely contribute to the protective effects of CDC-exosomes, but does not suffice to confer comprehensive therapeutic benefit alone. Hence, other miRNAs (i.e. miR-22, miR-24) in the repertoire may exert synonymous or perhaps synergistic effects

Table 1
Pathologic effects of exosomes in the heart.

Exosome sources	Key findings (function of exosomes)	Cargo responsible for effect	Refs
Neonatal rat cardiac fibroblasts	Promote cardiomyocyte hypertrophy	miR-21*	[68,133]
Goto-Kakizaki (GK) rat cardiomyocytes	Inhibit myocardial endothelial cell promotion, migration and tube formation	miR-320	[67]
Platelets from patients with sepsis	Induce endothelial cell apoptosis and cardiac dysfunction	NADPH, NOS, PDI	[92,93]
16K PRL-treated HUVECs	Reduce metabolic activity in cardiomyocytes	miR-146a	[69]
Heart and plasma from patients with acute peripartum cardiomyopathy	miR-146a-enriched exosomes are associated with dilated with cardiomyopathy	miR-146a	[69]

with miR-146a. Overall, the data provided by Ibrahim et al. [125] indicate that CDC-exosomes contain rich signaling information, and specifically the miRNAs transferred by these exosomes, that is capable of producing regeneration in a murine model of myocardial infarction (MI), and confer the same benefits as CDCs without transplantation of living cells.

4.4. Ischemic-preconditioned (IPC) exosomes mediate cardioprotection

It is well appreciated that ischemic preconditioning (IPC) elicits strong protective effects against cardiac ischemia/reperfusion (I/R)-induced injury [126,127]. However, the underlying mechanism is still not completely understood. Recently, Giricz et al. [52] showed that coronary effluent collected from IPC rat hearts contained more extracellular vesicles (EVs, microvesicles and exosomes) than effluent from control hearts, as evidenced by Western blotting. Furthermore, they observed that infarct size was significantly smaller in hearts perfused with coronary effluent collected from IPC hearts than those hearts perfused with coronary effluent from control hearts. Of interest, infarct size in hearts perfused with EV-depleted IPC coronary effluent was similar to controls. Therefore, the study by Giricz et al. [52] indicates that microvesicles/exosomes released from IPC hearts are responsible for the transmission of protective signals in the heart. It is important to note here, a recent study by Li et al. [128] showed that cardioprotection elicited by remote ischemic preconditioning (rIPC) was associated with the elevation of circulating miR-144, which was not included in plasma microparticles/microvesicles (50–400 nm), but incorporated with Argonaute 2. Nonetheless, they observed that the hairpin precursor miR-144 had a 4-fold increase in the exosome pellet derived from rIPC plasma [128]. Similarly, Jeanneteau et al. [129] recently observed that microvesicles derived from the blood of animals that underwent hind limb ischemia/reperfusion failed to decrease myocardial infarct size in rats. Put together, these studies suggest that exosomes rather than microvesicles (microparticles) contribute to the propagation of cardioprotective signals. Further studies will definitely be needed to elucidate the mechanisms underlying the IPC-exosome-mediated cardioprotection, using a clinically relevant in vivo model.

Recently, Feng et al. [130] performed a microRNA array for profiling the miRNAs in exosomes released from ischemic-preconditioned mesenchymal stem cells (MSCs). These MSCs were isolated from mouse bone marrow and subjected to two cycles of anoxia (30 min)–reoxygenation (10 min) as ischemic preconditioning (IPC). Feng et al. [130] observed that the levels of miR-22 were 4.5-fold higher, accompanied by miR-21 (3.3-fold), miR-210 (3.1-fold), miR-199a-3p (2.8-fold), and miR-24 (2.5-fold) in exosomes collected from IPC-MSCs (Exo^{IPC}) than control exosomes ($Exo^{non\text{-}IPC}$). Importantly, in vivo treatment of mice with Exo^{IPC} by direct injection into infarcted hearts resulted in a significant reduction of cardiac fibrosis and apoptosis, compared with $Exo^{non\text{-}IPC}$-treated hearts. Furthermore, these authors identified that the Exo^{IPC}-mediated protective effects are largely associated with transfer of exosomal miR-22 to surrounding cells. However, whether these preconditioned exosomes contain other beneficial proteins or ligands/receptors remains unclear.

5. Conclusions and future studies

Over the past decade, exosomes have been extensively studied as miRNA-carriers, as biomarkers for diagnosis of disease, and as a critical tool for cell-to-cell communication. However, their physiological and pathological roles in cardiovascular diseases are still vague. As reviewed above, exosomes derived from different conditions may contain different functional factors, which decide their properties; either harmful or beneficial (Tables 1 & 2). Most studies consistently implicate that disease messages may be spread around the body through exosomes (pathological role). But, we do not know whether specific blockade of

Table 2
Protective effects of exosomes in the heart.

Exosome sources	Key findings (function of exosomes)	Cargo responsible for effect	Refs
Human $CD34^+$ cells; Shh-modified $CD34^+$ cells	Promote angiogenesis and reduce infarct size in mice after acute myocardial infarction	miR-126, miR-130a, Shh protein	[111,114]
Human ES9.E1-derived MSCs	In myocardial ischemic–reperfused mice, exosome-treated animals exhibited significant preservation of left ventricular geometry and contractile performance during 28 days follow-up.	GAPDH, PGK, PGM, ENO, PKm2, phosphorylated PFKFB3	[102,103]
Human cardiac progenitor cells (CPCs)	Inhibit cardiomyocyte apoptosis and improve cardiac function after injection of exosomes into rat infarcted hearts.	miR-210, miR-132, miR-146a-3p	[134]
Human fetal heart-derived progenitor cells	Enhance the migration of human microvascular endothelial cells (HMECs), using in vitro scratch wound assay.	MMPs and EMMPRIN	[115]
Human cardiosphere-derived cells (CDCs)	Injection of exosomes into injured mouse hearts recapitulates the regenerative and functional effects produced by CDC transplantation.	miR-146a, miR-22, miR-24, miR-210	[125]
Mouse mesenchymal stem cells subjected to two cycles of anoxia (30 min) and re-oxygenation (10 min) (IPC).	In vivo delivery of IPC exosomes ameliorates fibrosis and reduces infarction sizes in mouse myocardial infarction model.	miR-21, miR-22, miR-24, miR-199a-3p, miR-210	[130]
Mouse mesenchymal stromal cells	In the murine model of hypoxic pulmonary hypertension, intravenous delivery of exosomes inhibits vascular remodeling and hypoxic pulmonary hypertension.	miR-17 superfamily, miR-204	[104]
Rat hearts upon three cycles of global ischemia (5 min) and reperfusion (5 min, IPC) in Langendorff mode.	Exosomes collected from perfusates of IPC hearts attenuate infarction size in non-preconditioned recipient hearts.	N/A (not available)	[52]

exosome production during disease is able to have therapeutic effects. Additionally, while it is well appreciated that stem cell-derived exosomes have therapeutic potential in cardiovascular disease, the in vivo mechanisms underlying the exosome-elicited action are not clear yet. Of interest, Yuan et al. [131] recently observed that only certain miRNAs wrapped in exosomes/microvesicles were efficiently transferred to recipient cells, which raises the possibilities that the transfer of miRNAs is selective and may be regulated by proteins located at either exosomes or recipient cells. Future studies will be needed to test this hypothesis. Moreover, the ability of exosomes to transfer lipid, RNA, and proteins raises very exciting possibilities for future therapeutic uses. For example, based on the regulatory effects of miR on therapeutic proteins [132,133], we could engineer host cells to overexpress a specific miR that is capable to be delivered via exosomes. These engineered cells can be either encapsulated to provide sustained local delivery or utilized as "factory" to produce therapeutic exosomes. The latter may be used as viral-free or cell-free agents in replacement of viral vectors or intact cells, which offers a safer approach for clinical application. It is also possible that the pathologic exosomes can be re-uploaded and be able to replace the harmful factors with beneficial ones. Overall, further understanding of pathways involved in exosome biogenesis, exosomal cargo loading, exosome secretion and exosome uptake will be of fundamental importance to address how to alter the quality and quantity of exosomes, as well as therapeutic efficacy for the treatment of cardiovascular disease.

Conflict of interest

The authors confirm that this article content has no conflict of interest.

Acknowledgements

The research in Dr. Guo-Chang Fan's lab is supported by NIH grant 2R01-087861.

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