HHS Public Access

Author manuscript

Pharmacol Ther. Author manuscript; available in PMC 2023 May 01.

Published in final edited form as:

Pharmacol Ther. 2022 May ; 233: 108025. doi:10.1016/j.pharmthera.2021.108025.

Extracellular Vesicles in Cardiovascular Disease: Biological Functions and Therapeutic Implications

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Abstract

Extracellular vesicles (EVs), including exosomes and microvesicles, are lipid bilayer particles naturally released from the cell. While exosomes are formed as intraluminal vesicles (ILVs) of the multivesicular endosomes (MVEs) and released to extracellular space upon MVE-plasma membrane fusion, microvesicles are generated through direct outward budding of the plasma membrane. Exosomes and microvesicles have same membrane orientation, different yet overlapping sizes; their cargo contents are selectively packed and dependent on the source cell type and functional state. Both exosomes and microvesicles can transfer bioactive RNAs, proteins, lipids, and metabolites from donor to recipient cells and influence the biological properties of the latter. Over the last decade, their potential roles as effective inter-tissue communicators in cardiovascular physiology and pathology have been increasingly appreciated. In addition, EVs are attractive sources of biomarkers for the diagnosis and prognosis of diseases, because they acquire their complex cargoes through cellular processes intimately linked to disease pathogenesis. Furthermore, EVs obtained from various stem/progenitor cell populations have been tested as cell-free therapy in various preclinical models of cardiovascular diseases and demonstrate unequivocally encouraging benefits. Here we summarize the findings from recent research on the biological functions of EVs in the ischemic heart disease and heart failure, and their potential as novel diagnostic biomarkers and therapeutic opportunities.

Keywords

Extracellular vesicle; Exosome; MicroRNA; Cell-cell communication; Cardiovascular; Therapeutics; Diagnosis

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Declaration of Competing Interest

The authors declare that there are no conflict of interest.

1. Introduction

Extracellular vesicles (EVs) are lipid-bilayer membranous particles secreted from all cell types in organisms from bacteria to humans and plants (Deatherage & Cookson, 2012; O'Brien, Breyne, Ughetto, Laurent, & Breakefield, 2020; Robinson, Ding, & Jiang, 2016). They are broadly divided into two major categories, exosomes and ectosomes (also called microvesicles). Exosomes are formed in the multivesicular endosomes (MVEs; or multivesicular bodies, MVBs) as intraluminal vesicles (ILVs) and secreted into extracellular space upon fusion of MVBs with the cell membrane (Baietti, et al., 2012; van Niel, D'Angelo, & Raposo, 2018), whereas microvesicles are generated by direct outward budding of plasma membrane of the cell. Thus exosomes and microvesicles have same membrane orientation identical to that of the cell surface, but are different in size (exosomes at 30–150 nm vs. ectosomes at 50–2000 nm in diameter) (Raposo, et al., 1996; Raposo & Stoorvogel, 2013) and cargo compositions (RNAs, proteins, lipids, and other metabolites) (Yanez-Mo, et al., 2015; Zaborowski, Balaj, Breakefield, & Lai, 2015). Once secreted, EVs navigate in the local tissue microenvironment or travel in large body fluids to distant organs (Meldolesi, 2018), where they interact with or enter target cell to trigger cellular phenotypic changes (Mulcahy, Pink, & Carter, 2014) (Meldolesi, 2018). In a decade, EVs have been in the limelight of research interest for their promising and continuously discovered roles in physiology and pathology as signaling mediators, biomarkers, and potential therapeutic agents (Y. Zhang, Liu, Liu, & Tang, 2019).

The heart is a sophisticated muscular pump that, through rhythmed contraction and relaxation, maintains normal blood pressure and nutrients/oxygen supplies to the entire body. Inter-cellular communication and coordination among different types of cardiac cells are essential for the integrity and proper function of the organ. The spontaneous contraction and relaxation of cardiomyocytes are tightly controlled by rhythmogenic and conductive cells and neuronal system. Coronary blood vessels, comprised of vascular endothelial cells (ECs), smooth muscle cells (VSMCs), and perivascular connective tissues, supply oxygen and nutrients to meet the metabolic need of the heart. Fibroblasts provide additional mechanical and electrophysiological support, whereas monocytes and macrophages clear damaged tissue and maintain homeostasis. The inter-cellular communications in the heart are mediated through cell-cell contact, cell-matrix interaction, and extracellular biochemical signals (G. Chen, et al., 2018; Pluchino & Smith, 2019). In last decade, EVs have been increasingly recognized as potential significant autocrine and paracrine communicators by delivering molecular cargoes from source cells to neighboring cells or distant organs and participating in the programming of cardiac microenvironment (Poe & Knowlton, 2018; Sluijter, Verhage, Deddens, van den Akker, & Doevendans, 2014).

2. The biology of EVs

2.1. EV biogenesis

The biogenesis of EVs has been extensively studied, as discussed in excellent recent reviews (Mathieu, Martin-Jaular, Lavieu, & Théry, 2019; O'Brien, et al., 2020; van Niel, et al., 2018). Exosomes are formed by inward budding of the limiting membrane of endosome into the lumen, as ILV, giving the multivesicular appearance of the endosome, thus the

term “MVE” or “MVB” (Colombo, Raposo, & Théry, 2014; Klumperman & Raposo, 2014) (average diameter, MVB ~386 nm, ILV ~50 nm; average number, ~22 ILVs per MVB (Hyenne, Labouesse, & Goetz, 2018; Ostrowski, et al., 2010). The process is driven by the cargoes – primarily the internalized plasma membrane of the endosome and the recruited *trans*-Golgi network (TGN) – that form microdomains on the limiting membrane, and is executed by the ESCRT (ESCRT-0, -I, -II and –III) complexes, including ESCRT-accessory molecules, ALIX (ALG-2 interacting protein X), TSG101 (tumor susceptibility gene 101 protein), and VPS4 (vacuolar protein sorting-associated protein 4), which contribute to the stepwise vesiculation (i.e., cargo segregation, membrane budding and fission, and vesicle release into MVBs) (Kajimoto, Okada, Miya, Zhang, & Nakamura, 2013; Trajkovic, et al., 2008). Alternatively, exosomes can be formed in an ESCRT-independent manner that requires ceramide for the generation of membrane microdomains and cargo sorting (Hurley, 2008, 2015), and tetraspanin family proteins (CD63, CD81, CD82 and CD9) have also been shown to regulate ESCRT-independent sorting of cargoes to ILVs (future exosomes) (van Niel, et al., 2015; van Niel, et al., 2011). Microvesicles, on the other hand, are generated through direct outward budding of the plasma membrane, and the process shares certain core features of stepwise vesiculation seen in the exosome formation, thus involving some of the same machineries (Colombo, et al., 2014). For example, the ESCRTs are also found in the microvesicles (Hurley, 2015) and required for the generation of microvesicles (Wehman, Poggioli, Schweinsberg, Grant, & Nance, 2011), whereas TSG101 and the ATPase VPS4 were reported to participate in the scission and release of microvesicles (Nabhan, Hu, Oh, Cohen, & Lu, 2012). In addition, cholesterol is abundant in both exosomes and microvesicles and crucial for their respective pathways of biogenesis (Del Conde, Shrimpton, Thiagarajan, & Lopez, 2005; Mobius, et al., 2003).

Importantly, the releases of both exosomes and microvesicles rely on cytoskeletal components (actin and microtubules), the associated molecular motors (dynein, kinesins and myosins) and molecular switches (small GTPases) (Hsu, et al., 2010; Ostrowski, et al., 2010; Sinha, et al., 2016). MVEs are transported along microtubules to the plasma membrane (Mittelbrunn, Vicente Manzanares, & Sanchez-Madrid, 2015), and their docking at the plasma membrane is supported by branched-actin filaments (Sinha, et al., 2016); RAS-related protein (RAB) GTPases, especially RAB27 isoforms, play essential roles in MVE transport and docking at the plasma membrane to promote fusion (Ostrowski, et al., 2010). The fusion of MVBs with the plasma membrane and subsequent release of ILVs are regulated by SNAREs, a family of proteins primarily involved in the regulation of membrane fusion and exocytosis (Fader, Sanchez, Mestre, & Colombo, 2009; Jahn & Scheller, 2006). Similarly, the release of microvesicles requires their fission from the plasma membrane, a process that is dependent on the interaction of actin and myosin interaction and ATP-dependent contraction (McConnell, et al., 2009). The activation of small GTP-binding proteins including ARF6 and ARF1 leads to the phosphorylation of myosin light chain (MLC) and actomyosin contraction, resulting microvesicles budding out from the membrane (Muralidharan-Chari, et al., 2009; Sedgwick, Clancy, Olivia Balmert, & D'Souza-Schorey, 2015).

2.2. EV cargoes

EVs carry both membrane and soluble cargoes, which are characterized by specific sets of lipids, proteins, nucleic acids (RNA and DNA), and metabolites (Jeppesen, et al., 2019; Murillo, et al., 2019). Both exosomes and microvesicles have the same membrane orientation. The lipid compositions of their membranes resemble that of raft microdomains, with enriched cholesterol, sphingomyelin, phosphatidylserine, and ceramide, relative to plasma membrane (Skotland, Sandvig, & Llorente, 2017). Protein markers for exosomes include the ESCRT machinery proteins (Alix, TSG101, VSP40), Syntenin-1 (Kugeratski, et al., 2021), heat-shock proteins (HSP70), and transmembrane tetraspanins (CD63, CD9, and CD81), although these may also exist in microvesicles at much lower abundance (Jeppesen, et al., 2019; Kowal, et al., 2016). EV carried cell-type specific proteins, especially membrane surface proteins, have been very useful for identifying and isolating cell-type specific EVs (Loyer, et al., 2018). The cargo nucleic acids are particular interesting due to their potential as effective mediators of inter-tissue communication. In fact, the functional significance of EV-mediated transfer of miRNA and mRNAs to target cells has been well established (Skog, et al., 2008; Valadi, et al., 2007). EVs acquire majority of biotypes of RNAs and RNA fragments from source cells (Nolte-'t Hoen, et al., 2012; Valadi, et al., 2007). For example, miRNA, tRNAs, mRNA and fragmented mRNAs, lncRNAs, and tRNAs are all found in exosomes, although evidence also suggest that the overall concentrations of RNAs in EVs are relatively low (Chevillet, et al., 2014; Li, et al., 2014).

Unlike membrane cargo, sorting of RNA cargoes into EVs is not well understood. RNAs can be loaded into EVs passively, due to their abundance in the cytosol (Mateescu, et al., 2017), but active sorting is likely the dominant mechanism, as exosomes contain unique sets of RNAs that differ from those of their parent cells (Chaput & Thery, 2011). RNA loading has been associated with their binding affinity for the lipids at microdomains where EV bud into MVB (for exosomes) or extracellular space (for microvesicles). For example, ceramide, produced by neural sphingomyelinase 2 (nSMase2) during EV formation, promote miRNA sorting into exosomes (Kosaka, et al., 2013). In addition, a number of RNA-binding proteins (RBPs, including Argonaute, annexin A2, MVP [major vault protein], HNRNPA2B1 [heterogeneous nuclear ribonucleoproteins A2/B1], YBX1, SYNCRIP and lupus La protein) that bind particular sequence motifs in the RNA or recognize unique secondary RNA structures have been suggested to contribute to RNA loading (Garrus, et al., 2001; Gibbings, Ciaudo, Erhardt, & Voinnet, 2009). In addition, different machineries have been proposed to perform specific nucleic acid sorting, including the ESCRT-II subcomplex that could act as an RNA-binding complex (Irion & St Johnston, 2007), and the tetraspanin-enriched microdomains that could sequester RNA-binding proteins in the membrane subdomains (Perez-Hernandez, et al., 2013). Finally, miRNAs contain certain motifs, such as GGAG, UGAG, CCCU, or UCCU that can be recognized by sumoylated heterogeneous nuclear ribonucleoproteins (hnRNPs), are overrepresented in the exosomes (Villarroya-Beltri, et al., 2013).

2.3. EV release and intercellular communication

Upon release from source cells, EVs navigate in the extracellular fluid, lodge on matrix around cells and near intercellular junctions, travel to adjacent tissues areas (Nawaz &

Fatima, 2017), or enter large fluids (i.e., blood, lymph, and cerebral spinal fluids) to reach remote organs (Meldolesi, 2018). The functional impact of EVs is dependent on the state of source cells, which dictates the biologic property of EVs, and on the local tissue microenvironment. For example, EVs released from activated inflammatory cells can carry active enzymes on surface and cause profound adverse tissue remodeling and disease pathology (Genschmer, et al., 2019; Wiesner, El Azzouzi, & Linder, 2013). However, generally, EVs transfer biological signals to target cells via multiple modes depending on the proteins and lipids on the surfaces of both the EV and target cell (Figure 1). For example, EVs can interact with target cells through membrane bound ligand-receptor pairs to trigger intracellular signaling. The EV membrane can fuse with plasma membrane and release cargo contents directly into cytoplasm. The EVs can be internalized into target cells via endocytic pathways, include macropinocytosis, phagocytosis, clathrin-mediated endocytosis, caveolin-dependent endocytosis, and clathrin/caveolin-independent endocytosis (Mulcahy, et al., 2014) (Bang & Thum, 2012); once internalized in the endosomes, EVs can back fuse with endosome membrane to release bioactive cargo contents to cytoplasm or degrade through lysosomal system (Joshi, de Beer, Giepmans, & Zuhorn, 2020; Le Blanc, et al., 2005). Nevertheless, mechanisms of EV cargo release inside target cells remain largely unclear.

2.4. EV isolation

The current standard technique for EV isolation is differential centrifugation with ultracentrifugation (serial centrifugations to remove cells and cell debris, followed by ultracentrifugation to pellet EVs) (Thery, Amigorena, Raposo, & Clayton, 2006), which may be combined with density gradient ultracentrifugation to remove non-EV proteins and EV subpopulations (Iwai, Minamisawa, Suga, Yajima, & Shiba, 2016; Jeppesen, et al., 2019). However, size-based isolation techniques are being increasingly used (ultrafiltration (Cheruvanky, et al., 2007) and TFF system (Heinemann, et al., 2014) that may also be combined with size exclusion chromatography (Boing, et al., 2014). Polymer-based precipitation methods (PEG precipitation) are also commonly used especially when the samples sizes are small (M. Cheng, et al., 2019). The EV can also be isolated by affinity-based capture (immunoaffinity capture such as using anti-EpCAM and anti-CD63 (Tauro, et al., 2012). Readers are referred to an excellent recent review wherein detailed EV isolation methods and challenges are discussed (Gardiner, et al., 2016; Sahoo, et al., 2021). Nevertheless, no isolation method is based on EVs' origin of generation. The EVs used in most studies are mixture of exosomes, small microvesicles, and potentially other EVs, which poses one of the greatest challenges for all the aspects of EV research (Ha, Yang, & Nadithe, 2016).

2.5. EV characterization

As recommended in the guidelines for the minimal information for studies of extracellular vesicles (MISEV2018) (Thery, et al., 2018), the identification of exosomes should include the following 4 parameters: 1) the size distribution and the yield by nanoparticle tracking analysis (NTA) or dynamic light scattering (DLS), 2) morphology and presence of a bilayer by high-resolution electron microscopy or single particle analyzer, 3) and at least one transmembrane or cytosolic protein marker by western blot, flow cytometry, or other

universal biomarker-based detection method, 4) degree of purity reflected by a negative marker (Thery, et al., 2018). After characterizing the basic features of exosomes, more specific detection of exosomes from certain cells or under a pathological state will be applied, such as the content of certain miRNA, lipid or protein and its function in physiology or pathology.

2.6. EV heterogeneity

Beyond the lack of isolation method or definitive markers to differentiate between exosomes and small microvesicle and the fact that different cell types or same cell-type at different functional states produces EVs of different cargo contents (Cocucci & Meldolesi, 2015; Kalluri & LeBleu, 2020), EVs are intrinsically heterogenic. This is because even within a single cell or a single MVB, ILVs (future exosomes) can be formed via different mechanisms (e.g., ESCRT dependent or independent), which results in the generation of exosomes carrying different cargoes. Despite intensive research, it is still unclear which sub-species of vesicles is responsible for a given effect. Thus we should also take this fascinatingly complex feature of EVs into consideration, no matter it is the nanoparticle characterization or therapeutic development. Importantly, studies of applying EVs in the *in vivo* animal models use a wide range of doses, some of which may not be physiologically relevant. Thus, it is likely that many findings will continue to be refined and validated.

3. EVs in ischemic heart disease

Ischemic heart disease, including myocardial infarction (MI) and consequent heart failure, is the leading cause of death worldwide (Virani, et al., 2021). MI is caused by occlusion of a coronary artery (White & Chew, 2008), depriving cardiomyocytes of oxygen and nutrients (Barile, Moccetti, Marban, & Vassalli, 2017; Kanamori, et al., 2011). As the result, cell death (apoptosis, pyroptosis and necrosis) occurs within 20 min, and complete necrosis occurs at 2-4 h after persistent coronary arterial occlusion (Thygesen, et al., 2007). The catastrophic event triggers a series of reparative responses, including collateral formation, neovascularization, and inflammation (Prabhu & Frangogiannis, 2016). Cardiomyocyte death and inflammatory stimuli also induce a pro-fibrotic response in resident fibroblasts, which secrete extracellular matrix (ECM) proteins (Sun, et al., 2004). And prolonged inflammation and fibrotic scare expansion contribute to irreversible adverse remodeling, arrhythmogenicity, and ultimately the development of heart failure (Alibhai, Tobin, Yeganeh, Weisel, & Li, 2018; Frangogiannis, 2017). Exosomes isolated from the plasma of healthy volunteers protect the myocardium from ischemic reperfusion (I/R) injury in animals (Vicencio, et al., 2015), and those obtained from the pericardial fluids of patients receiving aortic valve surgery promote angiogenesis in the ischemic limb injury (Aday, et al., 2021), suggesting a physiologically protective role of EVs in the body fluids. ILVs contained in the MVBs and EVs in the extracellular spaces have been observed directly in cardiac tissues (Sahoo & Losordo, 2014); and upon ischemic injury, the numbers of small EVs (sEVs, ~118nm) and large EVs (lEVs, ~252 nm) in the infarcted mouse heart, primarily released from cardiomyocytes and ECs, are significantly increased, by 6-20 folds and 10-30 folds, respectively (Loyer, et al., 2018). These evidence highlight the potential significance of EVs in the pathophysiology of ischemic injury, repair, and remodeling (Sahoo & Losordo, 2014).

3.1. Cardiomyocyte-derived EVs (^{CM}EVs)

Cardiomyocytes are not typical secretory cells, but they secrete EVs (Xu, Ye, Song, & Huang, 2019). When cultured under hypoxic or ischemia-mimetic environment or isolated from ischemic heart, cardiomyocytes release EVs (^{CM}EVs) with apparently altered cargo contents (proteins , miRNAs , lncRNAs , and circRNAs ; Table 1). These bioactive cargoes act on other cardiac cells, including cardiomyocytes, ECs, fibroblasts, and monocytes/macrophages, affecting cellular functions related to cell death, inflammatory response, neovascularization, and fibrosis (Malik, et al., 2013; J. Yang, et al., 2018; Y. Yang, et al., 2016; C. Zhang, Wu, Xu, Potter, & Gao, 2010). Among the protein cargoes, heat shock protein 60 (HSP60), previously shown to cause cardiomyocyte damage via TLR4 signaling (Kim, et al., 2009), is enriched in EVs from cardiomyocytes with hypoxic re-oxygenation treatment (Gupta & Knowlton, 2007), thus may potentially mediate cardiac ischemic reperfusion injury. Cx43, a ventricular cardiomyocyte gap junction protein previously shown to present in the ^{CM}EV membrane and involved in the delivery of heterogeneous DNAs and uptake of EVs (Soares, et al., 2015) to reduce the cardiomyocyte toxicity of anti-cancer drugs (Martins-Marques, et al., 2016); MI leads to downregulation of Cx43 in cardiomyocytes and ^{CM}EVs, thus may potentially reduce the protective effects of EVs (Martins-Marques, et al., 2020).

The ^{CM}EVs derived from hypoxic/ischemic cardiomyocytes have consistently shown to promote angiogenesis and inflammation. ^{CM}EVs promote EC proliferation, migration, tube-like-structure formation, and angiogenesis, which are attributable to cargo miRNAs (miR-222, −143, and 27a, 28-3p, 34a, miR-22 and miR-143) (Ribeiro-Rodrigues, et al., 2017; J. Yang, et al., 2018) and circRNA (circHIPK3) (Y. Wang, et al., 2020) that upregulate the expression of angiogenic genes (Garcia, Ontoria-Oviedo, Gonzalez-King, Diez-Juan, & Sepulveda, 2015) or MMPs in ECs (Table 1). Paradoxically, ^{CM}EVs from hyperbaric oxygen-treated cardiomyocytes also promote angiogenesis through a mechanism involving elevated lncRNA MALAT concentration in the ^{CM}EVs (Shyu, Wang, Fang, Pan, & Lin, 2020). Loyer et al found that lEVs, primarily derived from cardiomyocytes and ECs, activate pro-inflammatory response in Ly6C+ cardiac monocytes (Loyer, et al., 2018). Notably, ^{CM}EVs obtained from MI patients can induce macrophage activation and a proinflammatory profile (Almeida Paiva, et al., 2019). Interestingly, inhibition of miR-19a-3p in ^{CM}EVs can de-repress HIF-1α expression and promotes EC proliferation and survival (Gou, Xue, Tang, & Fang, 2020).

The effects of hypoxic/ischemic ^{CM}EVs on fibroblasts, however, appear less consistent among different studies; ^{CM}EVs have been shown to promote fibrotic response through cargo miRNA-195 and −208 (Morelli, Shu, Sardu, Matarese, & Santulli, 2019; J. Yang, et al., 2018) and lncRNA ENSMUST00000122745 (in sEV) and Neat1 (in 1EVs) (Kenneweg, et al., 2019), but also shown to attenuate fibrotic response through cargo miRNA-30d, lncRNA AK139128 (J. Li, et al., 2021; L. Wang & Zhang, 2020), and protein HSP-60 (Gupta & Knowlton, 2007) (Fig. 2).

Furthermore, our group investigated the systemic effects of ^{CM}EVs. It had been known that cardiomyocyte-specific miRs (i.e., myo-miRs, including miR-1, 133, 208 and 499) are rapidly and markedly increased in the peripheral blood of patient with acute MI (Goren, et

al., 2012), we found that these myo-miRs are carried primarily in the circulating ^{CM}EVs, which transfer myo-miRs into bone marrow mononuclear cells, suppressing chemokine receptor CXCR4 expression to mobilize progenitor cells (M. Cheng, et al., 2019). Thus, ^{CM}EVs released from infarcted heart can act as “SOS” signal to induce a systemic reparative response.

3.2. Endothelial cell-derived EVs (^{EC}EVs)

ECs are another important source of EVs during ischemic heart disease (Loyer, et al., 2018; Radecke, et al., 2015). In STEMI patients and MI mice, miR-126 is enriched in ^{EC}EVs, which are released into circulation, promoting splenic monocyte mobilization and MI repair (Akbar, et al., 2017), whereas lncRNA LINC00174 in ^{EC}EVs suppresses cardiomyocyte autophagy and apoptosis induced by ischemia-reperfusion (Su, et al., 2021). In ECs, overexpression of HIF-1 leads to increased miR-126 and 210 cargoes in ^{EC}EVs, promoting Sca1+ cardiac progenitor cells (CPCs) survival and angiogenesis (Ong, et al., 2014), whereas overexpression of KLF2 induces miR-24-3p cargo in ^{EC}EVs, suppressing CCR2 expression and the pro-inflammatory activity of monocytes and reducing cardiac inflammation (Qiao, et al., 2020).

3.3. Macrophage ($^{M\Phi}$EVs) and fibroblast (^{FB}EVs) derived EVs

Notably, MI injury is associated with inflammation and fibrosis. The M1-like macrophage-derived EVs (M1EVs) carry high level of miR-155, which aggravates MI-induced tissue damage by downregulating multiple target genes, including RAC1 (Rac family small GTPase 1), PAK2 (p21 [RAC1]-activated kinase 2), Sirtuin 1, and AMPKα2 (protein kinase AMP activated catalytic subunit alpha 2), in ECs to inhibit angiogenesis (S. Liu, et al., 2020) and by downregulating SOS1 (son of sevenless 1) and SOCS-1 (suppressor of cytokine signaling 1) in fibroblasts to inhibit fibroblast proliferation (C. Wang, et al., 2017). The M2-like macrophage EVs mediate myocardial fibrosis after acute MI (Y. Wang, et al., 2021). In addition, EVs obtained from cardiac fibroblasts (^{FB}EVs) following treatment with hypoxic re-oxygenation can mimic the benefit of ischemic post-conditioning through miR-423-3p (H. Luo, et al., 2019). Furthermore, telocytes, another type of stromal cells with long and thin prolongations, secrete EVs that ameliorate MI by delivering miR-21-5p (Liao, et al., 2021).

Clearly, studies dissecting the biological effects of cardiac EVs have begun to gain novel insights into the paracrine mechanism in cardiac ischemic injury and repair. Nevertheless, it is notably that cargo contents of EVs derived from same cell types vary among different experimental platforms, and discrepancy exists in the effects of ^{CM}EVs on cardiomyocyte survival and fibroblast activity. This may reflect the fact that EVs are highly heterogeneous populations, and EV cargo contents heavily depend on the culture conditions and functional states of source cells. Importantly, most of the studies used EVs isolated from *in vitro* cultured cells, therefore may not accurately reflect the characteristics of EVs secreted by the respective cell types *in vivo* in the ischemic myocardium. It is possible that the seemly contrasting effects may reflect cardiomyocytes at various degrees of stress, such as those in the infarct vs. boarder areas. Thus, it would be necessary to dissect the contents of EVs and associated biogenic processes in temporal and spatial manner in the ischemic myocardium.

Lastly, the functional outcome of endogenous cell-type specific EVs on ischemic heart disease have not been reported, and new development of technology that permit tracking and isolating tissue-specific EVs would aid the progress in this direction (W. Luo, et al., 2020; Verweij, et al., 2019).

4. EVs in cardiac hypertrophy and heart failure

Pathological cardiac hypertrophy is characterized by cardiomyocyte enlargement, interstitial fibrosis, and vascular insufficiency, which collectively leads to decompensating cardiac dilation, systolic and diastolic dysfunction, and heart failure (Nakamura & Sadoshima, 2018; Waldenstrom & Ronquist, 2014). During adverse remodeling, cardiomyocyte hypertrophy is associated with deregulated electrophysiology and metabolism, whereas cardiac fibroblasts convert to activated myofibroblasts, proliferating and producing access extracellular matrix (Nakamura & Sadoshima, 2018; Takeda & Manabe, 2011). Vascular insufficiency precipitates the process to heart failure. The inter-cellular communications, via paracrine factors, direct cell-cell interactions (e.g., gap junctions), and cell-extracellular matrix interactions, play critical roles in the pathogenesis of cardiac hypertrophy and heart failure (P. Zhang, Su, & Mende, 2012).

Strong evidences suggest that under pathological condition, cardiac fibroblast-derived EVs transfer miRNAs into cardiomyocytes, directly promoting cardiomyocyte hypertrophic growth (Table 2). In an early study, Bang et al (Bang, et al., 2014) revealed that cardiac fibroblasts secrete exosomes that contain high abundance of the passenger strand miRNAs (“star” miRNAs). Specifically, miR-21* is transported into cardiomyocytes, where it targets SORBS2 (sarcoplasmic protein sorbin and SH3 domain-containing protein 2) and PDLIM5 (PDZ and LIM domain 5), contributing to the cardiac hypertrophy in a pressure overload model (Bang, et al., 2014) (Fig. 2). Similarly, Tian C et al found miR-27a*, another star miRNA abundantly expressed in fibroblast-derived exosomes, is also transported into cardiomyocytes and suppresses PDLIM5 expression, worsening cardiac hypertrophy in an MI-induced chronic heart failure model (C. Tian, Hu, Gao, Hackfort, & Zucker, 2020). Furthermore, treatment of cardiac fibroblasts with angiotensin II (Ang II), another inducer of cardiomyocyte hypertrophy, also alter the compositions of exosomes, which in turn increases Ang II production, its receptor expression, and the renin angiotensin system in cardiomyocytes, worsening the pathology of cardiac hypertrophy (Lyu, et al., 2015). Notably, reactive oxygen species and oxidative stress play a significant role in cardiomyocyte dysfunction, myocardial hypertrophy, and fibrosis in chronic heart failure (CHF). Nrf2 (Kelch-like ECH-associated protein 1-nuclear factor erythroid 2-related factor 2) is a major transcription factor of the antioxidant defense mechanism. In a rat model of post-MI CHF (6 weeks), three microRNAs, microRNA-27a, 28-3p, and 34a were identified highly expressed in the left ventricle (C. Tian, Gao, Zimmerman, & Zucker, 2018). Further mechanistic studies revealed that these miRNAs are preferentially incorporated into exosomes produced from TNF-α–stressed cardiomyocytes and fibroblasts, which mediate their inter-cellular exchange to repress Nrf2 translation and anti-oxidant gene expression, as a potential mechanism contributing to ischemic heart failure (C. Tian, et al., 2018).

^{CM}EVs have also been shown to transport miRNAs into fibroblasts and modulate fibrosis. For example, miR-378, abundantly expressed in the mammalian heart as a suppressor of cardiomyocyte MAPK pathway and hypertrophy (Ganesan, et al., 2013), has recently been shown by Yuan J et al to be carried in ^{CM}EVs and transferred into fibroblasts, where it targets MKK6, attenuating p38 MAPK phosphorylation, the p38 MAPK-Smad2/3 pathway, fibroblast proliferation and secretion of collagen 1a1, 3a1, and MMP9 (Yuan, et al., 2018). In contrast, miR-217, upregulated in the heart of patients with CHF, can also be transferred by ^{CM}EVs to fibroblasts, exacerbating fibrosis (Nie, et al., 2018). Thus, ^{CM}EVs can carry miRNAs that suppress or increase fibroblast activities. Interestingly, lncRNA-H19, elevated in the exosomes of infarcted myocardium, attenuates inflammation and increases cardiomyocyte survival during the phase of cardiac remodeling (Hobuß, et al., 2020), whereas miR-155, carried in the exosomes from hypertrophic cardiomyocytes, aggravetes inflammation of macrophages via MAPK pathway (Yu, Qin, Peng, Bai, & Wang, 2020). However, the *in vivo* effects on the structure and function of hypertrophic and failing hearts remain to be determined.

In endothelial cells, the Ang II signaling has been linked to the microparticle formation via AT1 receptor/NADPH oxidase/Rho kinase targeted to lipid rafts (Burger, et al., 2011). In an isoproterenol-induced cardiac hypertrophy model, endothelial microparticles modulate the proinflammatory effects through a mitochondrial related mechanism to attenuate cardiac hypertrophy (Tripathi, et al., 2019).

Interestingly, in uremic heart, infiltrating macrophages expresses miR-155, which is loaded into exosomes and transferred into cardiomyocytes via membrane fusion, where it directly targets FoxO3a to increase cardiomyocyte pyroptosis and hypertrophy of uremic cardiomyopathy (B. Wang, et al., 2020).

5. Diagnostic potentials of EVs

An early and accurate diagnosis of the onset of cardiovascular diseases is critical for the timely therapeutic intervention. For AMI, current diagnostic tools include electrocardiogram (ECG) and a serum panel of biomarkers, creatinine phosphokinase-muscle band (CPK-MB), troponin-T (TnT), and troponin I (TnI). Upon AMI, myocardial injury leads to rapid appearance of cardiac TnT and TnI in the blood, the detection of which is widely used for AMI diagnosis owing to its high sensitivity and specificity. However, troponin levels do not begin to rise until 4–6 h after infarction (Skeik & Patel, 2007). Furthermore, TnI is not specific for cardiac damage after MI, since the elevated levels could also be observed in cardiotoxicity following chemotherapy, chronic renal failure, pulmonary embolism and after non-cardiac surgery (Klein Gunnewiek & van de Leur, 2003; Korff, Katus, & Giannitsis, 2006). Thus, there is a need for additional biomarkers to aid fast and specific diagnosis for AMI. The biogenesis of EVs is intrinsically linked to the cellular process of disease, therefore their complex components, in combination, may potentially better reflect disease pathology than any individual molecular markers. In addition, the bilayer membrane of EVs, formed via raft-like microdomains enriched in cholesterol, sphingolipids, and ceramides to offer strong protection and preservation of cargo materials (such as RNAs) in the body fluids. These features, in addition to their easy access and analysis, make them attractive

biomarkers for diagnosis and prognosis of diseases in clinic, as demonstrated in serial studies of cardiovascular disease.

The four myo-miRs (miR-1, 133a/b, 208a and 499) are specifically enriched in cardiomyocytes and involved in the regulation of heart development and function. In patients with AMI, their levels in the plasma are markedly increased within 1-24 h and peaked in 3-6 h post-AMI (M. Cheng, et al., 2019; Goren, et al., 2012), well correlated with the change of cardiac TnT and cardiomyocyte damage and necrosis (Goren, et al., 2012). Notably, miR-133a and miR-208b levels are strongly associated with the risk of death (Widera, et al., 2011). A study from our lab reveals that these circulating myo-miRs, released from ischemically injured myocardium, are carried in exosomes of patients with STMI but not those without acute coronary syndrome (M. Cheng, et al., 2019) (Table 3). In addition, ischemic heart disease patients with severe and extensive coronary narrowing often need bypass surgery, which also induce the release of cardiac EVs into peripheral circulation, and the levels of miR-1 and 133a/b in the circulating EVs are significantly increased within 24 h and 48 h after the operation (Yellon & Davidson, 2014).

Nevertheless, these myo-miRs do not predict future cardiovascular events or indicate the existence of chronic heart failure (Matsumoto, et al., 2013; Widera, et al., 2011). Interestingly, elevated levels of p53-responsive miRNAs, including miR-192, 194, and 34a, in the circulating EVs of AMI patients at “subacute phase” (around 18 days after MI) predict the development of heart failure in 1 year, and specifically, the levels of miR-194 and 34a are correlated with left ventricular end-diastolic dimension (Matsumoto, et al., 2013). Furthermore, in patients with systolic heart failure, the levels of miR-423-5p, 320a, 22, and 92b, are elevated in serum exosomes and correlate with important clinical prognostic parameters (Goren, et al., 2012). In stable coronary artery disease (CAD) patients, above-median levels of miR-126 and 199a in the FACS-sorted plasma microvesicles from ECs (CD31+/CD42b−) and platelets (CD31+/CD42b+) is associated with a reduced risk of major adverse cardiovascular events with 6-years follow-up (Jansen, et al., 2014). Thus, miRNA cargoes of circulating EVs may be effective biomarkers for diagnosis and prognosis of acute and chronic cardiovascular disease.

The protein cargoes of EVs may also be used as biomarkers to assess cardiovascular risk or pathology. EC injury and apoptosis is a hallmark of CAD (Katz, et al., 2005) and EC apoptosis is characterized by membrane lipid rearrangement, especially the exposure of phosphatidylserine from inner leaflet to outer leaflet, which bind to apoptosis-related Annexin V. In a clinical study with 6 years follow-up for major adverse cardiovascular and cerebral event (MACCE)-free survival, a high level of circulating microparticles that bind to Annexin V and display EC marker CD31 (i.e., $CD31^+$/Annexin V^+) have been identified to be positively associated with a higher risk for cardiovascular death and serve as an independent predictor for cardiovascular events in patients with stable CAD (Matsumoto, et al., 2013).

6. Therapeutic potentials of stem/progenitor cell-derived EVs

EVs acquire the load of bioactive molecules from parent cells and exert biological activity, to certain extent, reflective of their cellular origin. A number of unique features make them ideal as cell-free therapeutic agents or drug carriers. The strong membranous structure serves to protect and preserve bioactive cargoes from degradation and withstand various routes of administration. They are able to efficiently pass biological barriers, including the blood brain barrier, and enter into cells. Unlike living cells, EVs are small and easily obtained (from body fluids or cell-culture media), modified (chemically and biophysically), and stored and display low immunogenicity *in vivo*. As a result, EVs from various sources have been evaluated extensively in animal models for treatment of ischemic heart disease, including MI and I/R injury. Encouraging results have been shown with EVs derived from various populations of stem/progenitor cells, including endothelial progenitor cells (EPCs), mesenchymal stem cells (MSCs), cardiac progenitor cells (CPCs), cardiosphere-derived cells (CDCs), embryonic (ESCs) and induced (iPSCs) pluripotent stem cells and their differentiated cardiovascular cells (El Harane, et al., 2018; Erbs, et al., 2007; Gallet, et al., 2017; Gao, et al., 2018; Losordo, et al., 2011; Menasché, et al., 2015; Menasché, et al., 2018; Steinhoff, et al., 2017; Tang, et al., 2009; van Berlo, et al., 2014). EVs often demonstrate equal benefit as their source cells (Bian, et al., 2014; Gallet, et al., 2017).

6.1. EPC-derived EVs (^{EPC}EVs)

The early work from Sahoo et al demonstrated the potential of exosomes produced by human CD34+ EPCs for treatment of cardiovascular disease (Sahoo, et al., 2011; Sahoo & Losordo, 2014). The authors showed that these exosomes mediate the proangiogenic paracrine activity in the ischemic limb tissue (Mathiyalagan, et al., 2017). Khan et al further demonstrated that ^{EPC}EV administration into infarcted heart increases neovascularization, inhibit cardiomyocyte apoptosis, reduce scar size, and improves left ventricular function, and that IL-10 is critical for ^{EPC}EVs' beneficial effects by repressing aberrant activation of cardiac inflammation (Yue, et al., 2020). In addition to targeting ECs, ^{EPC}EVs were also found to enhance vascularization by acting on fibroblasts to promote fibroblast-to-endothelial transition (Huang, et al., 2021; Ke, et al., 2021).

6.2. MSC-derived EVs (^{MSC}EVs)

The EVs derived from MSCs (^{MSC}EVs) of different origin (i.e., bone marrow, adipose, umbilical cord, and heart) have demonstrated exclusive beneficial effects (i.e., attenuating cardiomyocyte death, augmenting neovascularization, and reducing inflammation, fibrosis, and infarct size) in MI and I/R injury models across rodents and large mammals. The benefits have been attributed to EVs acting on different cardiac cell types via transfer of bioactive miR, lncRNA, and protein cargoes (Lai, et al., 2010) (Table 4). For example, ^{MSC}EVs act on cardiomyocytes to reduce autophagy (G. Chen, Wang, Ruan, Zhu, & Tang, 2021), apoptosis (Han, et al., 2019; J. Liu, et al., 2018; Peng, Zhao, Peng, Xu, & Yu, 2020), ferroptosis (Song, et al., 2021), and pyroptosis (Mao, Liang, Zhang, Pang, & Lu, 2019). ^{MSC}EVs secreted from both innate and hypoxia-treated MSCs carry a high concentration of miR-125-5p that, after transfer into cardiomyocytes, increase the contractile function and reduce autophagy flux and apoptosis, leading to reduced infarct size (C. Xiao, et al.,

2018; L. P. Zhu, et al., 2018). ^{MSC}EVs also enhances cardiac repair by acting on ECs to increase neovascularization (Ju, et al., 2018; T. Ma, et al., 2018; N. Wang, et al., 2017), on cardiac fibroblasts to promote VEGF secretion and angiogenesis (Q. Li, et al., 2021), and on cardiac macrophages to promote M2 polarization and reduce pro-inflammatory cell infiltration (Deng, et al., 2019; R. Xu, et al., 2019; Zhao, et al., 2019).

6.3. CDC and CPC derived EVs (^{CDC}EV and ^{CPC}EV)

^{CDC}EV and ^{CPC}EV have been extensively evaluated for treating MI and I/R injury (Barile, et al., 2017; Gallet, et al., 2017; A. G. Ibrahim, Cheng, & Marban, 2014; van Berlo, et al., 2014). Serial studies from Marban group have shown that ^{CDC}EVs increase ischemic cardiac repair by acting on cardiomyocytes to increase proliferation and reduce apoptosis (Cambier, et al., 2017; A. G. Ibrahim, et al., 2014), on macrophages to reduce infiltration and macrophage-mediated cardiomyocyte apoptosis (de Couto, et al., 2017), on fibroblast to promote SDF1 and VEGF secretin and angiogenesis (Tseliou, et al., 2015). Importantly, in porcine models of acute and chronic MI, the authors confirm that ^{CDC}EVs reduce scarring, attenuate adverse remodeling, and improve function (Gallet, et al., 2017; A. G. E. Ibrahim, et al., 2019). In addition, CPC-derived exosomes have also shown to afford substantial beneficial effects on cardiac protection by alleviating cell apoptosis and attenuating adverse remodeling (L. Chen, et al., 2013; J. Xiao, et al., 2016).

6.4. ESC and iPSC derived EVs (^{ESC}EVs and ^{iPSC}EVs)

Pluripotent stem cell-derived EVs provide a cell-free system that uses the immense regenerative power of these cells while avoiding the risks associated with direct cell transplantation and teratoma formation. Indeed, EVs derived from these cells and their differentiated cardiac cells have shown strong potential (Chandy, et al., 2020; Jung, Fu, & Yang, 2017). Khan et al found that mouse ESC-derived exosomes enhanced neovascularization, cardiomyocyte survival, and reduced fibrosis post infarction, consistent with resurgence of cardiac proliferative response (Khan, et al., 2015). The findings are further supported by a recent study using EVs isolated from human ESC-derived cardiovascular progenitor cells (Wu, et al., 2020). Intriguingly, Adamiak et al compared the ability of mouse ^{iPSC}EVs and their source iPSCs and found ^{iPSC}EVs are safer and more effective for cardiac repair, as indicated by further improved left ventricular function, superior perfusion, and ameliorated apoptosis and hypertrophy, over the effect of iPSCs (Adamiak, et al., 2018). The Yang group evaluated the effects of exosomes from human iPSC-derived cardiomyocytes in a rodent MI model, and found increased cardiomyocyte re-entry into cell cycle, upregulated autophagy flux that led to reduced apoptosis and fibrosis and augmented repair (Jung, et al., 2021; Santoso, et al., 2020). Interestingly, they observed larger size mitochondria enriched in these exosomes that appear to aid restoring cellular bioenergetics (Ikeda, et al., 2021).

7. Enhancing EV therapeutics

The therapeutic benefit of native stem cell EVs can be further enhanced by optimizing their bioactive cargoes, tissue targeting, uptake and cargo release (de Abreu, et al., 2020). These can be achieved by modulating source cells via culture microenvironment (e.g., hypoxia),

genetic (mRNA or miRNAs or siRNAs) or metabolic engineering or by directly modifying EVs via chemical or physiological approaches (Armstrong, Holme, & Stevens, 2017).

7.1. EV bioactivity

It is known that exposing cells to ischemic/hypoxic environment can activate protective response. Such treatments have been shown to increase beneficial bioactive cargoes in stem cells, such as in MSCs (miR-210, 125b-5p, and lncRNA-UCA1), CPCs (hypoxia-inducible therapeutic covariant miRNA clusters), and ESCs-derived cardiovascular progenitor cells (LncRNA-MALAT1) (H. Cheng, et al., 2020; L. Sun, et al., 2020; Wu, et al., 2020; J. Zhu, et al., 2018) (Table 4). Cells can also be conditioned with drugs, and treatment of MSCs with Atorvastatin led to increased lncRNA-H19 in ^{MSC}EVs (P. Huang, et al., 2020). Additionally, the source cells can be engineered, i.e., via introduction of nucleic acids (mRNAs, miRNAs, siRNAs, DNAs, or virus) or proteins. The therapeutic effectiveness of EVs have shown to be enhanced by transduction of CPC with miR-322 and 133a and transduction of MSCs with miR-150 and 101a, HIF-1a, CXCR4, SDF-1α, macrophage migration inhibitory factor (MIF), Akt, SIRT1, and TIMP2, and GATA4 (Gong, Liu, Wang, Liang, & Wang, 2019; Hao, et al., 2020; H. Huang, et al., 2020; Izarra, et al., 2014; K. Kang, et al., 2015; J. Ma, et al., 2017; Ni, et al., 2019; Ou, et al., 2020; J. Sun, et al., 2020; J. Wang, et al., 2020; Youn, et al., 2019; Zhu, et al., 2021) (Table 4). Genetic engineering not only provide genes or gene products to be packed into EVs, but also EV biogenesis mediators that can enhance the efficiency and selectivity of cargo loading (Sterzenbach, et al., 2017). Furthermore, EVs themselves can also be modified using chemical or physical methods to aid loading of synthetic bioactive materials in the EV membrane or cytosol. The exciting advances in the field of smart EV engineering for therapeutic applications have recently been reviewed elsewhere (Armstrong, et al., 2017).

7.2. EV biodistribution and tissue-specific targeting

EVs can be delivered to the heart via intramyocardial, intracoronary, and systemic (intravenous) routes. After systemic injection, EVs are cleared quickly from blood circulation and observed most abundantly in the liver, followed by lungs, kidneys, and spleen, and that detection peaked in the liver and kidney in the first hour after administration, while distribution to the lungs and spleen peaked between 2–12 h (M. Kang, Jordan, Blenkiron, & Chamley, 2021). This suggest that most of EVs may be taken up by non-cardiac cells, and that the EV concentrations in the un-injured and perhaps also chronically injured cardiac tissue may be low (O. P. Wiklander, et al., 2015; Yi, et al., 2020). Marban group compared the effects between catheter-based intracoronary delivery and open-chest intramyocardial delivery of exosomes in a porcine MI model; the results were in favor of intramyocardial delivery in functional parameters, such infarct size, adverse remodeling, and heart contractile function (Gallet, et al., 2017). Nevertheless, systemic administration is less invasive, thus safer, and applicable to patients with AMI. Our recent study, using bioorthogonal chemical labeling of EV proteome, demonstrate that cardiac acute ischemic injury dramatically increases the homing of systemically administered EVs (E. Zhang, et al., 2021), thus, EVs, like stem cells (Luger, et al., 2017), are attracted to the site of acute injury. We further hypothesize that modifying EV tropism may alter the biodistribution and achieve tissue-specific delivery, thereby enhancing local effective dose

and reducing potential systemic side effects. To this end, we have successfully engineered an ischemic myocardium targeting peptide (IMTP) fused to Lamp2b displayed on the exosome surface; the IMTP-exosomes are rapidly internalized into hypoxic H9C2 cells *in vitro* and display enhanced targeting in the ischemic myocardium (X. Wang, et al., 2018). Surface modifications can also be achieved chemically. Vandergriff et al conjugated CPC-derived exosomes with cardiac homing peptide (CHP) through a dioleoylphosphatidylethanolamine N-hydroxysuccinimide (DOPE-NHS) linker and demonstrated an increasing retention of the CHP-exosomes within the ischemia/reperfusion injured heart tissue (Vandergriff, et al., 2018).

7.3. EV uptake and cargo release

Effective cargo delivery into the target cell is one of the most significant steps in EV-mediated biological effects. However, the ability of cardiovascular cells to take up EVs and the mechanisms by which the internalized EVs release cargoes to the cytoplasm are still not well understood (Joshi, et al., 2020; Le Blanc, et al., 2005). Most of the internalized EVs are sorted into endolysosomal pathway, therefore likely undergo degradation (Fig. 1) (Heusermann, et al., 2016; Mulcahy, et al., 2014; T. Tian, et al., 2013). Thus it is likely that lysosomal escape be a point of manipulation for enhancing cytosolic EV cargo release. This can be achieved by equipping the nanoparticles with materials that increase endosome pH value (pH buffering or proton sponge), fusogenic peptide coating that disrupts endosomal membrane, or core-shell gel that swells to break the endosome (Cupic, Rennick, Johnston, & Such, 2019). For example, Nakase et al found that coating EVs with cationic lipids and pH-sensitive fusogenic peptides or with arginine-rich cell-penetrating peptides significantly increases the quantity of effective EV cargoes in the cytosol (Nakase & Futaki, 2015; Nakase, et al., 2017; Nakase, Noguchi, Fujii, & Futaki, 2016).

7.4. Large animal studies

The therapeutic benefits of ^{MSC}EV, ^{CDC}EV, and $^{iPSC\ CM}$EV for ischemic heart disease have been largely confirmed in the relevant large animal models, including pigs and monkeys. Intramyocardial injection of EVs obtained from hypoxia-preconditioned monkey bone marrow MSC have shown to promote angiogenesis, by miR-486-5p mediated MMP-19 expression and VEGF secretion in fibroblasts, augment contraction function, and reduce infarct size (Q. Li, et al., 2021). A carefully crafted spray of MSC-derived exosomes in porcine MI model have shown to increase angiomyogenesis, reduce fibrosis and adverse remodeling, and improve contractile function (Yao, et al., 2021). Administration of human ^{CDC}EVs have been shown to increase LVEF, reduced scare size, fibrosis and remodeling and increase angiogenesis in porcine MI model (Gallet, et al., 2017) and intramyocardial injection of exosomes leads miRNA transfer into macrophages and mediates cellular post-conditioning in pig ischemic reperfusion model (de Couto, et al., 2017). Recently, Gao L et al confirm that exosomes secreted by hiPSC-derived cardiac cells improves myocardial recovery without increasing the frequency of arrhythmogenic complications for myocardial injury (Gao, et al., 2020). Although no direct comparison has been made about which source is preferable, current large animal studies seem to recapitulate the benefits observed in small animals. These valuable studies pave the way towards clinical translation of this promising therapy.

7.5. Clinical trials

The clinical trials involving EVs continue to grow in number. A search for “extracellular vesicle”, “exosomes”, or “microvesicles” registered at https://www.clinicaltrials.gov revealed over 300 trials as active. Most trials are for cancer treatment (Herrmann, Wood, & Fuhrmann, 2021; O. P. B. Wiklander, Brennan, Lotvall, Breakefield, & El Andaloussi, 2019). Only a small number are for biomarkers and treatment of cardiovascular disease, such as Allogenic Mesenchymal Stem Cell Derived Exosome in Patients with Acute Ischemic Stroke [NCT03384433]. A few completed trials, such as using MSC exosomes for coronavirus pneumonia or dendritic cell-derived exosomes for in patients with advanced non-small cell lung cancer after conventional chemotherapy, suggesting administrations of EVs through various routes are safety and showing encouraging potential efficacy. Nevertheless, the vast majority of trials are ongoing, and the results remain to be seen in the near future.

8. Conclusions

Significant progress have been made over the last decade in our understanding of the biology of EVs and their important role, especially as effective mediators of inter-tissue crosstalk, in cardiovascular physiology and pathology, and their tremendous potential for the diagnosis and therapy of disease. We now know more about the intracellular processes and molecular machineries underlying the generation of EVs, and appreciate better the complexity and heterogeneity of EV cargoes as an integral component of tissue microenvironment. EVs acquire a selected pool of bioactive molecules and appear to exert biological effects reflective of their cell origin, however, detailed dissections of the underlying molecular underpinnings have just begun. The pathways of EV biogenesis are unique and intimately linked to the networks of intracellular membrane transport vesicles, known to be involved in most cellular activities in health and diseases. Thus EVs are an independent biological identity in the arena of paracrine mechanisms that contribute to the protection, pathology, and repair of ischemic heart disease. Meanwhile, EVs are potential superior biomarkers that may aid the diagnosis and prognosis of disease. The EVs are small non-living nanoparticles, packed with load of bioactive cargoes within robust membrane. They can pass tight biological barriers (e.g., blood brain barrier) and enter the cell to deliver cargoes. They are easily obtained, stored, and highly modifiable, thus presenting exciting opportunities for cell-free therapy and drug delivery. And preclinical studies using stem/progenitor cell EVs for ischemic heart disease exclusively demonstrate benefits, and the results often show EVs as effective as their donor cells.

However, the EV research also has challenges. Currently, EVs are isolated primarily based on their size, density, and discrete surface markers, but not on their origin of generation. They are heterogeneous, and no method or specific marker is available to differentiate between exosomes and small microvesicles or between exosome subpopulations, which hampers the biological characterization and therapeutic standardization. Similarly, the biogenesis of exosomes and microvesicles share similar features (e.g., membrane vesiculation) and machineries with endoplasmic reticulum (ER) or *trans* Golgi network, no biogenic pathway-specific intervention, gain or loss of function, is currently available to aid

establishing the physiological roles of endogenous exosomes and/or microvesicles without disturbing cellular homeostasis. Relevant to the cardiovascular system, little is known how cardiovascular specific pathophysiology alters the fundamentals of EV biology beyond cargo profiling, how cardiac cell-type specific EVs mediate cell-cell communications in a temporal and spatial manner in the injury myocardium, and how therapeutic EVs can be engineered in ways that maximize the beneficial effects within the hypoxic/ischemic and inflammatory microenvironment. Advances in these areas will not only help reveal previous unknown mechanisms of cardiovascular disease but also potentially lead to novel therapeutics to improve the clinical outcome.

Acknowledgements

This work was supported by the National Institute of Health (R01 Grants# HL138990 and HL131110 to G.Q.); the American Heart Association (Grant# 19TPA34910227 to G.Q., Grant# 830472 to C.H, and 19IPLOI34760713 to J.Y.); and UAB (startup fund to G.Q.).

Abbreviations

AGO2	Argonaute RISC catalytic component 2
ALIX	ALG-2 interacting protein X
ARF6	ADP-ribosylation factor 6
BAV	bicuspid aortic valve
CAD	coronary artery disease
CDC	cardiosphere-derived cells
CHF	chronic heart failure
CM	cardiomyocytes
CPC	cardiac progenitor cell
CPK-MB	creatinine phosphokinase-muscle band
CVD	cardiovascular disease
CXCR4	CXC-chemokine receptor 4
DCM	diabetic cardiomyopathy
EC	endothelial cell
ECG	electrocardiogram
EPC	endothelial progenitor cell
FB	fibroblast
hnRNP	heterogeneous nuclear ribonucleoprotein

ILV	intraluminal vesicle
iPSC	induced pluripotent stem cells
ER	endoplasmic reticulum
ESE	early sorting endosome
EV	extracellular vesicle
lEV	large EV
lncRNA	long non-coding RNA
LSE	late sorting endosome
MI	myocardial infarction
MSC	mesenchymal stem cells
MVB	multivesicular body
MVE	multivesicular endosome
ncRNA	non-coding RNA
Nrf2	Kelch-like ECH-associated protein 1-nuclear factor erythroid 2-related factor 2
nSMase2	neural sphingomyelinase 2
sEV	small EV
SNARE	soluble N-ethylmaleimide-sensitive-factor attachment protein receptor
TGN	*trans* Golgi network
TnI	troponin I
TnT	troponin-T
TSG101	tumor susceptibility gene 101 protein
TSPAN	tetraspanin
VPS	vacuolar protein sorting-associated protein

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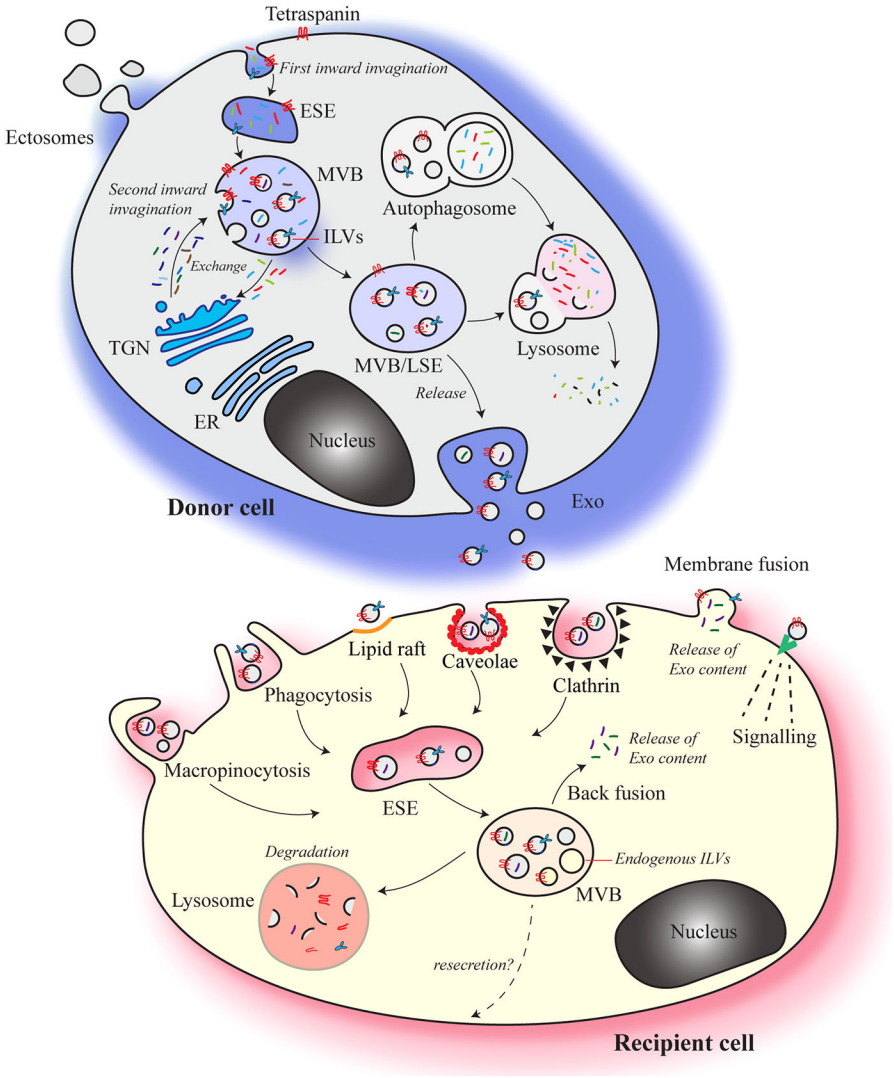

Fig. 1. The biogenesis, release and uptake of EVs.

Exosomes and ectosomes (microvesicles) are two major categories of EVs. Exosomes are formed as intraluminal vesicles (ILVs) in the multivesicular body (MVB), which exchanges vesicular cargoes with trans Golgi network or fuses with other early sorting endosomes (ESE). Protein, lipids and RNAs cargoes are sorted into ILVs during the biogenesis. Then MVB either fuses with cell membrane to release ILVs into the extracellular space as exosomes or fuses with autophagosomes or lysosomes for degradation. Microvesicles are generated by direct outward budding of the cellular plasma membrane. After released into the extracellular space, EVs impact the target cell via three modes. They can interact with the membrane receptors on the target cell to trigger intracellular signaling. They also can fuse with the cell membrane and directly release cargoes into cytoplasm. Lastly, the EVs can be internalized into target cells via endocytic pathways, include macropinocytosis, phagocytosis, clathrin-mediated endocytosis, caveolin-dependent endocytosis, and clathrin/caveolin-independent endocytosis. Once internalized, the exosomes within the endosome may be fusing with endosome membrane to release cargoes into the cytoplasm (back

fusion), sorted into lysosomes for degradation, or perhaps re-secreted into the extracellular space like endogenous exosome.

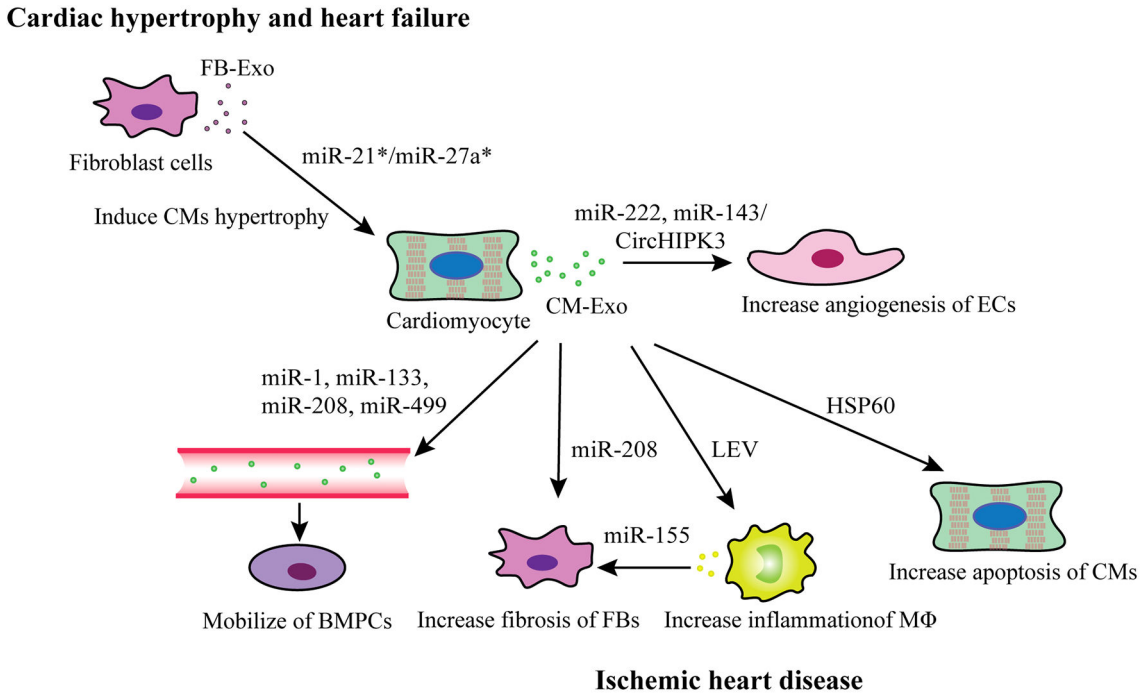

Fig. 2. EVs function in cardiovascular diseases.

During ischemic heart disease, cardiomyocyte (CM)-derived EVs (^{CM}EVs) transfer bioactive cargoes into local or remote cells and regulate their function, e.g., miR-222, 143 and circHIPK3 into endothelial cells (ECs) to promote angiogenesis, HSP-60 into cardiomyocytes to exacerbate apoptosis, miR-208 into fibroblasts (FB) to increase fibrosis, unidentified cargo into Ly6C+ monocytes to induce pro-inflammatory gene expression, and myo-miRs (miR-1, 133, 208, and 499) into remote BM cells to mobilize bone marrow progenitor cells (BMPCs). In addition, macrophage (MΦ)-derived EVs transport miR-155 into fibroblasts to increase fibrosis. During cardiac hypertrophy and heart failure, fibroblast-derived EVs transfer miR-21* and miR-27a* into cardiomyocytes, contributing cardiac hypertrophy.

Table 1

EVs mediate communication between cells in the prognosis of myocardial infarction

Origin	Cargo	Target gene / cells	Model	Function	Ref.
CM	Cx43	NA/ cardiac cells	Mice I/R	Cx43 into circulating EVs after MI↓	(Martins-Marques, et al., 2020)
CM	HSP-60	TLR4 / FB	NA (isolated rat CMs) [in vitro]	NFkB↑ p38↑ apoptosis↑	(Gupta & Knowlton, 2007)
CM	miR-30d	MAP4K4, integrin α5 / FB	Rat, mouse ischemic HF	FB proliferation↓	(J. Li, et al., 2021)
CM	miR-195	NA / FB	Mouse MI	FB differentiation into myo-FB↑	(Morelli, et al., 2019)
CM	ENSMUST00000122745 (sEV), Neat1 (lEVs)	NA / FB	Mouse MI	FB cell cycle ↑pro-fibrotic genes ↑Promote cardiac fibrosis ↑LVEF↓	(Kenneweg, et al., 2019)
CM	NA	iNOS, IL-1β, IL-6 / macrophages	EV from MI patients	macrophage activation↑pro-inflammatory profile ↑	(Almeida Paiva, et al., 2019)
CM	miRs	NA / EC	[in vitro]	proliferation↑ angiogenesis↑	(Garcia, et al., 2015)
$CM^{Ischaemia}$	miR-222, miR-143	MMP / EC	Mouse MI	ECs migration↑ capillary-like tub↑ sprouting formation ↑endothelial permeability ↑Neovascularization↑	(Ribeiro-Rodrigues, et al., 2017)
$CM^{Hypoxia}$	lncRNA AK139128	NA / FB	Rat MI	FB Apoptosis ↑Proliferation, Migration↓ and Invasion↓	(L. Wang & Zhang, 2020)
CM^{Hpoxia}; CM^{AngII}	miR-208	Dyrk2 / FB	Rat MI	FB proliferation↑ differentiation into myo-FBs↑ Collagen ↑	(J. Yang, et al., 2018)
$CM^{Hypoxia}$	circHIPK3	miR-29a / EC	Mouse MI	Angiogenesis↑ LVEF↑ infarct size↓	(Y. Wang, et al., 2020)
$CMs^{HperOxy}$	MALAT	miR-92a / myocardium	Rat MI	KLF2 and CD31 ↑infarct size↓	(Shyu, et al., 2020)
$CM^{anti\text{-}miR\text{-}19a\text{-}3p}$	Reduced miR-19a-3p	HIF-1α / EC	Mouse MI	EC proliferation↑ cell death↓	(Gou, et al., 2020)
EC/CM	1EV	NA/ monocytes	Mouse MI	IL -6, CCL 2, CCL7 from cardiac monocytes ↑local inflammation↑	(Loyer, et al., 2018)
EC	VCAM-1	NA / NA	subjects with PCI for stable CAD	infarct size↑ vascular inflammation↑	(Radecke, et al., 2015)
EC	LINC00174	SRSF1 / CM	Mouse I/R	CM autophagy ↓ CM poptosis↓	(Su, et al., 2021)
EC	miRNA-126-3p and - 5p	plexin-B2 / monocyte	Mouse MI, patience STEAMI	splenic monocyte mobilization↑	(Akbar, et al., 2017)
EC^{HIF1}	miR-126, miR-210	Spred1 / CPC	Mouse MI	Survival of Sca1+ CPCs ↑Angiogenic↑ genes oxygen consumption ↓	(Ong, et al., 2014)
EC^{KLF2}	miR-24-3p	CCR2 / monocyte	Mouse IR	migration of Ly6C high monocytes↓ Ly6C high monocyte recruitment↓	(Qiao, et al., 2020)
$EPC^{IL10\text{-}KO}$	ILK	NF-κB / EC	MI	NF-κB pathway ↑ inflammation↑	(Yue, et al., 2020)
$FB^{H/R}$	miR-423-3p	RAP2C / CM	Rat IR	CM viability↑ CM apoptosis↓	(H. Luo, et al., 2019)

Origin	Cargo	Target gene / cells	Model	Function	Ref.
M1Φ	miR-155	Sirt1/AMPKα2, RAC1-PAK2 / EC	Mouse MI	Mice survival↓ LVEF↓ LVFS↓ nitric oxide synthase↓ RAC1-PAK2 signaling pathways ↓Vessels↓	(S. Liu, et al., 2020)
M2Φ	CircUbe3a	miR-138-5p, RhoC / FB	Mouse MI	CFs proliferation↑CFs migration↑CFs phenotypic transformation↑fibrosis↑	(Y. Wang, et al.,2021)
MΦ	mir-155	Son of Sevenless 1 / FB	Mouse MI	CFs proliferation↑ Inflammation↓	(C. Wang, et al.,2017)
telocytes	miR-21-5p	cdip1 / EC	Rat MI	EC survival ↑LVEF↑LVFS↑infarct size↓fibrosis ↓angiogenesis↑	(Liao, et al., 2021)

CDC, cardiosphere-derived cells; cCFU-FB^{GATA4}, cardiac colony-forming unit fibroblasts (cCFU-FB) overexpressing GATA4; CM, cardiomyocytes; CMI, convalescent myocardial infarction; CPCs, cardiac progenitor cells; CSC, cardiac stromal cells; EC, endothelial cells; EPC, endothelial progenitor cells; FB, Fibroblast; H/R, hypoxia-reoxygenation; HyperOxy, Hyperbaric oxygen; IR, ischemia-reperfusion injury; LVEF, left-ventricular ejection fraction; LVFS, left-ventricular fractional shortening; MΦ, macrophage; M1Φ, M1macrophage; M2Φ, M2 macrophage; NA, not applicable; OE, over expression; PCI, percutaneous coronary interventions.

Table 2

EVs mediate communication between cardiac cells in cardiac hypertrophy

Cells origin	Cargo	Target gene	Model	Function	Ref.
CM	miR-378	MKK6/FB	Mouse TAC	p38 MAPK-Smad2/3 pathways ↓ cardiac hypertrophy↓ fibrosis↓	(Yuan, et al., 2018)
CM	miR-217	PTEN/FB	Mouse TAC/ transplant patients with CHF	heart size ↑heart weight/body weight radi↑cardiac hypertrophy ↑ fibrosis↑ LVEF↓	(Nie, et al., 2018)
CM	miR-155	ERK, JNK, p38/ Macrophages	Ang II-induced CM hypertrophy	Inflammation↑	(Yu, et al., 2020)
CM	lncRNA-H19	NF-κB signaling and VDR/ Cardiac cell, Mainly FB	Mouse MI	CMs survival↑ vitamin D receptor (VDR) ↓liver X receptor (LXR) ↓cardiac apoptosis inflammation↓	(Hobuß, et al., 2020)
CM and FB	miR-27a, miR-28-3p, and miR-34a	Nrf2 / cardiac cells	Rat MI, HF	oxidative stress↑Nrf2 translation↓	(C. Tian, et al., 2018)
$CM^{TNF\text{-}\alpha}$ $FB^{TNF\text{-}\alpha}$	Nrf2	CM and FB	Rat MI and CHF	antioxidant enzymes↓ oxidative stress↑	(C. Tian, et al., 2018)
$FB^{Ang\ II}$	Ang II	AT1R, AT2R /CM	Angiotensin II treated	Cardiomyocyte hypertrophy↑	(Lyu, et al., 2015)
FB	miR-21*	SORBS2, PDLIM5/ CM	TAC, Ang II Induced cardiac Hypertrophy	CMs hypertrophy↑ cardiac hypertrophy↑	(Bang, et al., 2014)
FB	miR-27a*	PDLIM5/ CM	Rat MI	cardiac hypertrophy↑hypertrophic gene↑ myocardial contractility↓	(C. Tian, et al., 2020)
FB	Ang II	AT1R/CM	Ang II–rat neonatal fibroblasts (in vitro)	Cardiomyocyte hypertrophic↑	(Lyu, et al., 2015)
EC	Not known	O(2)(−) and Rho kinase/ EC	Ang II–treated WT and $ApoE^{-/-}$ mice	microparticle release↑ROS formation↑ inflammation↑	(Burger, et al., 2011)
EC	MAPKAPK2	E-selectin and ICAM-1/ EC	Isoproterenol induced cardiac Hypertrophy	E-selectin↑ cytoskeleton rearrangement, monocyte attachment↑cardiac hypertrophy↑	(Tripathi, et al., 2019)
Macrophage	miR-155	FoxO3a/ CM	uremic mouse	CMs pyroptosis ↑cardiac hypertrophy↑fibrosis↑	(B. Wang, et al., 2020)

CM, cardiomyocytes; CHF, chronic heart disease; EC, endothelial cells; FB, fibroblasts; TAC, Transverse aortic constriction; Ang-II, Angiotensin.

Table 3

The EV biomarker on cardiovascular system

Disease	Origin	Biomarker	Timing	Study design	Biological Function	Isolation	Refs
AMI	Plasma (rat and human)	miR-1, miR-133a, miR-499, miR-208a	increased at 1–3 h, peaked at 3–12 h and decreased at 12–24 h after AMI	33 consecutive AMI and 33 non-AMI patients and rat MI model	Biomarker for AMI	NA	(Goren, et al., 2012)
AMI	Serum (mice and human)	miR-1	1-24 h after AMI, peaked at 6h, return to basal levels at 3 days after AMI	thirty-one patients with AMI and 20 healthy volunteers Rat AMI	Biomarker for AMI	NA	(Y. Cheng, et al., 2010)
AMI	Plasma (human)	miR-133a	0-72 h after AMI, peaked at 21.6 ± 4.5 h after the onset of AMI	13 AMI patients, 176 angina pectoris patients and 127 control subjects	Biomarker for AMI	NA	(F. Wang, et al., 2013)
AMI	Plasma (mice and human)	miR-1, miR-133a/b, miR-208a and miR-499	Peaked within 6 h after AMI	3 CAD patients with acute ST-elevation MI 3 CAD patients without acute syndrome; Mouse AMI	mobilize of BMPCs for cardiac	ExoQuick	(M. Cheng, et al., 2019)
HF post-AMI	Serum (human)	miR-192, miR-194, and miR-34a	18 days after AMI	21 HF patients, 65 control group	Predict LV end-diastolic dimension 1 year after AMI	ExoQuick	(Matsumoto, et al., 2013)
Systolic HF	Serum (human)	miR-423-5p, miR-320a, miR-22, and miR-92b	chronic stable, class C HF	30 patients with systolic HF and 30 volunteers	clinical prognostic for systolic HF patients	ExoQuick	(Goren, et al., 2012)
CABG	Plasma (human)	miR-1, miR-133, miR-210	24 h and even in 48 h post-CABG	pre-CABG and post-operation from 15 patience	CABG induce miRNA transfer	Exo-spin Mini-Columns	(Emanueli, et al., 2016)
CAD	Plasma (human)	miR-126, miR-199a	followed up for 6 years	181 stable CAD patients	predict CV events	Microvesicle sorted by flow cytometer	(Jansen, et al., 2014)
stable CAD	Plasma	CD31+/ Annexin V^+	fasting for ≥12 h, follow up 6 years	50 CAD patients	Predict risk in CAD	Affinity isolation	(Sinning, et al., 2011)

AMI, acute myocardial infarction; CABG, coronary-artery by-pass graft surgery; CAD, coronary artery disease; HF post-AMI, heart failure after acute myocardial infarction; HF, heart failure; NA, not applicable.

Table 4.

Stem cells derived EVs in ischemic heart disease

Origin	Cargos	Target	Model	Function	Isolation	Refs.
ESC	miR-294	undefined	Mouse MI	LVEF ↑ LVFS ↑ LVESD4↓ Neovascularization↑ CMs Proliferation↑ CMs Survival↑	UC	(Khan, et al., 2015)
ESC-CVPC^{Hypoxic}	LncRNA-MALAT1	miR-497/ CMs, ECs	Mouse MI	LVEF ↑ LVDS↓ Scar area↓ CM survival↑ angiogenesis↑	UC	(Wu, et al., 2020)
iPSC	Not known	undefined	Mouse I/R	apoptosis ↓ hypertrophy↓	UC	(Adamiak, et al., 2018)
iPSC-CM	Mitochondria	undefined	Mouse MI	contractile profile↑ cell survival↑ cardiac remodeling↓	UC	(Ikeda, et al., 2021)
iPSC-CM	miRs	Undefined/CMs	Mouse MI	CMs Survival ↑ LVEF↑ Autophagy↑	PEG8000	(Santoso, et al., 2020)
iPSC-CM^{Hypoxic}	miR-106a-363 cluster	Notch3/ CMs	Mouse MI	LVEF↑ LVEDV↓LVESV↓ CMs proliferation↑ CMs Survival↑	PEG-8000	(Jung, et al., 2021)
iPSC-CM, EC and SMC	Not known	undefined	Porcine MI	angiogenic ↑ LVEF↑	UC	(Gao, et al., 2020)
CDC	Not known	undefined	Porcine MI	LVEF↑Scar↓ fibrosis↓ remodelling↓ angiogenesis ↓ inflammation →	Ultrafiltration and PEG	(Gallet, et al., 2017)
CDC	Not known	Undefined / Fibroblast	Rat MI	SDF1 and VEGF secreted by FBs↑ Angiogenesis↑ remodeling↓	PEG	(Tseliou, et al., 2015)
CDC	miR-146a	IRAK, TRAF6, SMAD4, MPO/ CMs	Mouse MI	LVEF↑ Scar size↓ angiogenesis↑ CM proliferation↑ inflammation↓ apoptosis↓	ExoQuick (SBI)	(A. G. Ibrahim, et al., 2014)
CDC	miR-146a, miR-181b, and miR-126	protein kinase C δ/ Mφ	Rat and Porcine I/R	LVEF↑ infarct size↓ CD68+ macrophage(Mφ) ↓	PEG	(de Couto, et al., 2017)
CDC	Y RNA frgament	IL-10/CMs	Rat I/R	Infarct size↓ CD68+ Mφ ↓Apoptosis↓	Ultrafiltration and ExoQuick (SBI)	(Cambier, et al., 2017)
CDC$^{β\text{-catenin-active}}$	miR-92a	BMP2/ CMs	Mouse MI	LVEF ↑ LVFS ↑ LVESD↓ Fibrosis↓ remodeling↓	Ultrafiltration	(A. G. E. Ibrahim, et al., 2019)
CPC$^{miR\text{-}322\text{-}OE}$	miR-322	Nox2-derived ROS/EC	Mouse MI	Infarct size-4↓ Capillary density↑	UC	(Youn, et al., 2019)
CPC$^{miR\text{-}133a\text{-}OE}$	miR-133a	Undefined/CM	Rat MI	Fibrosis↓ Hypertrophy↓ vascularization↑ CMs proliferation↑	UC	(Izarra, et al., 2014)
EPC	miR-1246 and miR-1290	ELF5 and SP1/FB	Rat I/R	MET in CFs↑ Infarct size↓ fibrosis↓	UC	(Huang, et al., 2021)
EPC	miR-218-5p/ miR-363-3p	p53/JMY/FB	Rat I/R	CF Proliferation ↑angiogenesi↑ MET in CFs↑ Infarct size↓ fibrosis↓	UC	(Ke, et al., 2021)
EPC (CD34^{+})	Not known	Undefined/EC	mouse	angiogenesis ↑	Sucrose density gradient centrifuge	(Sahoo, et al., 2011)
MSC	proteins	Not known	Mouse I/R	Infarct sizes↓ Risk area↓	Sucrose density gradient centrifuge	(Lai, et al., 2010)
MSC	miR-125b-5p	P53/CM	Mouse MI	Fibrotic size↓ Autophagic Flux↓ LVEF↑ LVFS↑	UC	(C. Xiao, et al., 2018)

Origin	Cargos	Target	Model	Function	Isolation	Refs.
MSC	miR-132	RASA1/EC	Mouse MI	Neovascularization↑	exosome isolation reagent (Invitrogen)	(T. Ma, et al., 2018)
MSC	miR-143-3p	CHK2/CM	Rat I/R	Cell apoptosis↓ myocardial I/R injury↓	HieffTM Quick exosome isolation kit	(G. Chen, et al., 2021)
MSC	miR-182	TLR4/ macrophage	Mouse MI	LVEF↑ LVFS↑ infarct size↓ Macrophage M2 polarization↑	UC	(Zhao, et al., 2019)
MSC	miR-210	Efna3/EC	Mouse MI	Angiogenesis↑ LVEF↑ LVFS↑	UC	(N. Wang, et al., 2017)
MSC	miR-25-3p	FASL, PTEN, EZH2/CM	Mouse MI	infarct size ↓CM apoptosis↓ inflammation↓	Exosome Isolation Reagent (Thermo Scientific)	(Peng, et al., 2020)
MSC	miR-486-5p	Mmp19/FB	Mouse and monkey s MI	VEGF release from FB ↑ angiogenesis↑ LVEF↑ LVFS↑ infarct size ↓	UC	(Q. Li, et al., 2021)
MSC	lncRNA-KLF3-AS1	miR-138-5p/ Sirt1/CM	Rat MI	CMs pyroptosis↓ Heart weight/ tibia length↓ infarct size↓	exosome isolation kit (Invitrogen)	(Mao, et al., 2019)
MSC	Not known	undefined	Porcine MI	Fibrosis↓ angiomyogenesis ↑LVIDd↓LVIDs↓ LVFS↑ LVEF↑	Ultrafilter (3-kDa cutoff)	(Yao, et al., 2021)
MSC	Not known	AKT1/ AKT2// Macrophage	Mouse MI	inflammatory cells infiltration↓ CM apoptosis↓ macrophage M2 polarization↑	Density gradient ultracentrifugatio	(R. Xu, et al., 2019)
$MSC^{HIF\text{-}1\alpha\text{-}OE}$	HIF-1α	Undefined/E C	Rat MI	LVEF↑ LVFS↑ neovessel formation ↑	UC	(J. Sun, et al., 2020)
$MSC^{CXCR4\text{-}OE}$	CXCR4	Akt/CM	Rat MI	LVEF↑ LVFS↑ angiogenesis↑ infarct size↓	ExoQuick-TC	(K. Kang, et al., 2015)
$MSC^{SDF1a\text{-}OE}$ (human UC)	SDF1	PI3K/CM	Mouse MI	CM Apoptosis↓ CM Autophagy ↓Microvascular production↑	UC	(Gong, et al., 2019)
$MSC^{miR\text{-}150\text{-}OE}$	miR-150-5p	TXNIP/CM	Rat I/R	CM apoptosis ↓ Fibrosis ↓ infarct size ↓	ExoQuick-TC	(Ou, et al., 2020)
$MSC^{MiR\text{-}101a\text{-}OE}$	miR-101a	undefined	Mouse MI	Infarct size↓ LVEF↑ LVFS↑	UC	(J. Wang, et al., 2020)
$MSC^{Hypoxic}$	miR-210	nSMase2/EC, CM	Rat MI	angiogenesis↑ apoptosi↓ Rat survival after MI↑ LVEF↑LVFS↑ fibrotic scar ↓	UC	(J. Zhu, et al., 2018)
$MSC^{Hypoxic}$	miR-125b-5p	p53 and BAK1/CM	Mouse MI	CM apoptosis LVEF↑LVFS↑ ESCV↓ LVID↓ fibrosis area ↓	UC	(L. P. Zhu, et al., 2018)
$MSC^{Hypoxic}$	miR-210	AIFM3/CM	Rat MI	LVFS↑LVEF↑ LVIDd↓ LVIDs↓ infarct size↓	UC	(H. Cheng, et al., 2020)
$MSC^{Hypoxic}$	lncRNA-UCA1	miR-873-5p/ XIAP/CMs	Rat MI	CM survival ↑ LVEF ↑ LVFS↑Fibrosis area↓	Ribo™ Exosome Isolation Reagent	(L. Sun, et al., 2020)
$MSC^{Atorvastatin\text{-}treated}$	lncRNA-H19	VEGF, ICAM-1/ECs	Rat MI	CMs apoptosis↓ EC Migration↑LVEF↑ LVFS↑LVEDV↓ LVESV↓ infarct size↓ collagen↓	UC	(P. Huang, et al., 2020)
$cCFU\text{-}FB^{GATA4}$	GATA4, miR221	PTEN/ CMs	Mouse MI	(PI3K)/AKT signaling↓CMs apoptosis↓	UC	(Hao, et al., 2020)
$MSC^{Sirt1\text{-}OE}$ (adipose)	SIRT1	CXCL12 and Nrf2/EPCs	Mouse MI	Mice survival↑Vasculogenesis↑ infarct size↓ inflammation↓	UC	(H. Huang, et al., 2020)
MSC (adipose)	miR-93-5p	Atg7 or TLR4/CM	Rat MI	aumiddlehagy↓ inflammation↓ apoptosis↓	ExoQuick-TC	(J. Liu, et al., 2018)

Origin	Cargos	Target	Model	Function	Isolation	Refs.
MSCs (adipose)	Not known	S1P/SK1/ S1PR1/ Macrophage	Rat MI	macrophage M2 polarization↑apoptosis↓infarct size↓	UC	(Deng, et al., 2019)
MSC (cardiac)	Not known	undefined /EC, CM	Mouse MI	wall thickness↑ CM proliferation ↑angiogenesi↑	PEG 4000	(Ju, et al., 2018)
MSC (umbilical cord)	miR-23a-3p	DMT1/CM	Mouse MI	ferroptosis ↓ Infarct size ↓apoptosis↓	UC	(Song, et al., 2021)
MSCs (umbilical cord)	Not known	undefined	Rat MI	LVEF↑ LVFS↑ infarct size↓ inflammation↓ fibrosis↓apoptosis↓ angiogenesis↑	total exosome isolation kit (Life Technologies)	(Han, et al., 2019)
$MSC^{Akt\text{-}OE}$ (umbilical Cord)	Akt	PDGF-D/EC	Rat MI	LVEF↑ LVFS ↑LVID;d↓ LVID;s ↓angiogenesis↑	UC and density gradient centrifugation	(J. Ma, et al., 2017)
$MSC^{TIMP2\text{-}OE}$ (umbilical Cord)	TIMP2	Akt/Sfrp2/FB	Rat MI	Oxidative stress↓ Apoptosis↓ ECM remodeling↓	UC	(Ni, et al., 2019)
MSC^{MIF} (umbilical cord)	miR-133a-3p	AKT/EC, CM	Rat MI	LVEF↑ LVFS↑Apoptosis ↓fibrosis↓ angiogenesis↑ CM survival↑	Exosome Isolate kit (thermo fisher)	(Zhu, et al., 2021)

B2M, β2-microglobulin; CDCs, cardiosphere derived cells; CM, Cardiomyocytes; EC, endothelial cells; ESC, Embryonic stem cell; ESC-CVPCs, Embryonic stem cell derived cardiovascular progenitor cells; EPC, Endothelial Progenitor cells; iPSCs, induced pluripotent stem cells; iPSC-CM, induced pluripotent stem cells derived Cardiomyocytes; LVEF, left-ventricular ejection fraction; LVEDV, left-ventricular end-diastolic volumes; LVESV, left-ventricular end-systolic volumes; LVFS, left-ventricular fractional shortening; LVIDd, left ventricular internal diameter end-diastolic; LVIDs, left ventricular internal diameter end-systolic; MET, Mesenchymal to Endothelial Transition; MIF, Macrophage migration inhibitory factor; MSCs, mesenchymal stem cell; SBI, System Biosciences; SMC, smooth muscle cells; UC, Ultracentrifugation.