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Examining the Paracrine Effects of Exosomes in Cardiovascular Disease and Repair

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Cardiovascular disease is the leading cause of death worldwide, accounting for almost a third of deaths.[1] Although advances in research and cardiac care have increased survival, there is still a need to develop new strategies for prevention, intervention, and palliation, given the continued high-mortality rate. Extracellular vesicles (EVs) hold tremendous potential to monitor and treat cardiovascular disease because of their capabilities for analysis and modulation. EVs also compose a critical component of intercellular signaling by transporting molecular cargo from donor cells to recipient cells, thereby modulating gene expression and influencing the phenotype of the recipient cell.[2–7] Most studies have documented this intercellular communication in vitro, but emerging evidence suggests it also occurs in vivo.[8–11] In this review, we focus on the role of exosomes, a subset of EVs, as mediators of intercellular signaling in the heart. Exosomes are constitutively released from several cardiac cell types including cardiomyocytes, cardiac fibroblasts, endothelial cells, inflammatory cells, and resident stem cells[12] and may be responsible for the communication among these cell types under both physiological and pathological conditions.[13–15] Exosomes, when injected into the heart, are well known to mimic the cardioprotective and restorative properties of various progenitor or stem cells including cardiac progenitor cells (CPCs),[16] mesenchymal stem cells (MSCs),[17,18] hematopoietic stem cells,[19] and induced pluripotent stem cells (iPSCs).[20] Despite the importance of exosomes in paracrine signaling, less is known about the function of secreted exosomes and their paracrine actions among the different cell types of the heart.

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The iPSCs have evolved into a crucial model system to understand human cardiovascular disease. Protocols have been developed to differentiate patient-specific iPSCs into several cell types within the heart such as cardiomyocytes,[21–23] endothelial cells,[24,25] and smooth muscle cells.[26] As a result, iPSC-derived cell studies have elucidated mechanisms involved in the pathogenesis of long QT syndrome,[27] dilated cardiomyopathy,[28] dystrophic cardiomyopathy,[29,30] hypertrophy,[31] and diabetic cardiomyopathy,[32] and thus their exosomes may be useful in addressing critical questions regarding intercellular communication in cardiac function and disease. In this review, we discuss the role of exosomes in the understanding of cardiovascular disease as well as the potential of iPSC-derived cells to model the exosome-specific paracrine effects.

Nomenclature of EVs

The field of EV research has been hampered not only by lack of standardized nomenclature but also by lack of criteria to distinguish, isolate, and identify the different subtypes of EVs. The term *EV* encompasses several subtypes of generated cells and expelled vesicles that are enclosed by a membrane bilayer. These include exosomes, microvesicles, and apoptotic bodies (Figure 1). The subtypes can be differentiated by their size, content, and route of intracellular formation. Exosomes are between 25 and 200 nm in diameter and are formed inside multivesicular bodies within the endocytic pathway (Figure 2).[33] Here, the endosomal sorting complex required for transport facilitates the inward budding of the plasma membrane, which fills the exosome with cytoplasmic contents and retains membrane proteins specific to the cell of origin.[34–37] At this point, multivesicular bodies may either dock and fuse with the plasma membrane to release exosomes into the intracellular space or deliver their contents to the lysosome for degradation (Figure 2).[38,39] Microvesicles are 100 to 1000 nm in diameter and are formed by the outward budding and pinching of the plasma membrane,[40] whereas apoptotic bodies (>1000 nm) are much larger and are formed by apoptotic cell membrane blebbing.[41]

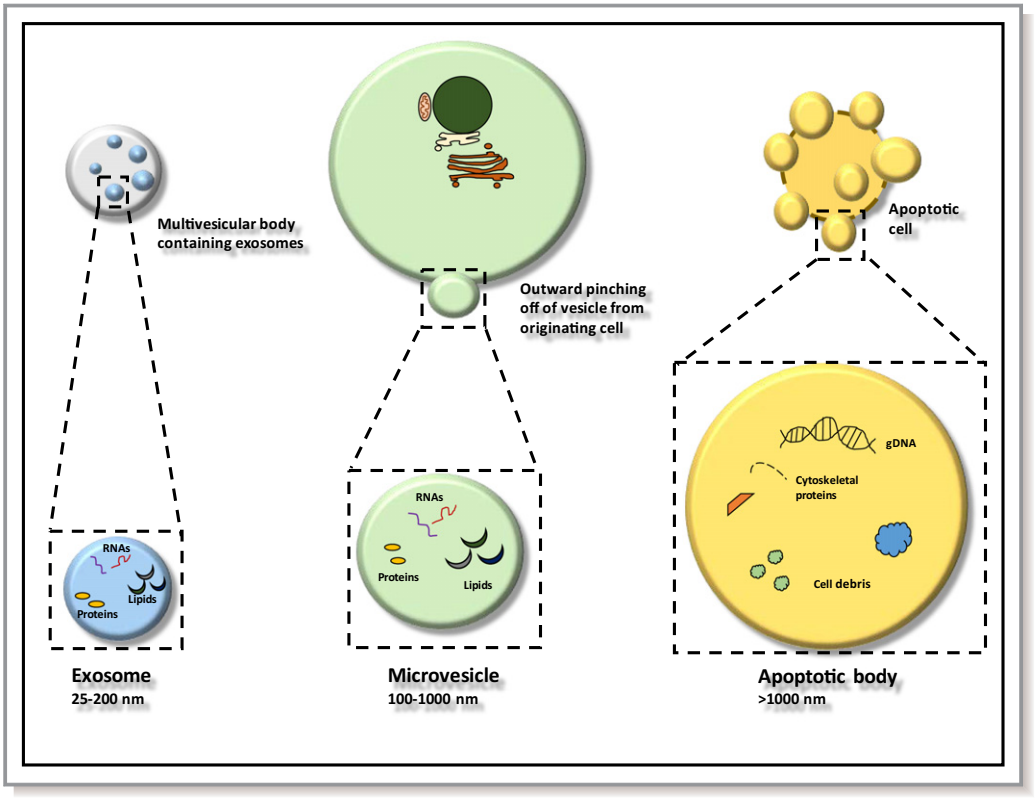

Figure 1. Extracellular vesicle subtypes. Extracellular vesicles are divided into 3 categories based on size, contents, and route of formation. Exosomes, the smallest, originate within multivesicular bodies and carry RNAs, proteins, and lipids. Microvesicles, the next largest, form through outward pinching off of the plasma membrane and also contain RNAs, proteins, and lipids. Apoptotic bodies are formed from dying cells as the plasma membrane blebs to recycle contents. Apoptotic bodies are variable in size and contain cell debris, genomic DNA (gDNA), and proteins.

Exosomal Composition

Exosomes contain lipids, proteins, RNAs, and microRNAs (miRs).[5,6] The composition of exosomes not only differs from microvesicles and apoptotic bodies[4] but also varies depending on cell type of origin, disease status, or stimuli during formation.[42–45] The exosome composition and internal cargo determine the outcome of the intercellular communication.[46] The field of exosome research is continuously expanding. Resources for obtaining up-to-date literature on exosome biology, roles in disease, and composition can be found in Table 1.

The lipid composition includes ceramide, sphingomyelin, phosphatidylcholine, and phosphatidylserine[47,48] within the membrane bilayer and cholesterol inside the exosomes. The organization of the lipids participates in the biological activity and can, in part, determine the fate of the exosome.[49] Exosome-specific proteins include the ubiquitously expressed tetraspanins CD81, CD9, CD63, CD82, HSP70 (heat shock 70-kDa protein), HSP60, and Alix (apoptosis-linked gene 2-interacting protein X); annexins and cytoskeletal proteins; and unique cargo proteins.[50,51] Moreover, exosomes contain RNAs and miRs that can change depending on the exosome subtype, cell type, or disease state.[34,52,53] The sorting of most proteins, RNAs, and lipids into exosomes is not well understood[46] and is an area of current investigation.

Exosome Uptake

Exosomes are internalized by their destination cells through multiple mechanisms. A potential route is endocytosis, which requires an initial surface-binding event either by direct interaction of a receptor with its ligand or through adhesion molecules such as integrins. Another route, membrane fusion of exosomes, has also been demonstrated in a minority of studies.[54,55] Other exosomal surface proteins such as CD47 may modulate the uptake process. For example, CD47, a thrombospondin 1 receptor, is present on the surface of EVs derived from Jurkat T cells and can alter gene expression and functional signaling in endothelial cells.[56,57] Whether other microenvironmental factors modulate exosome uptake is largely unknown.

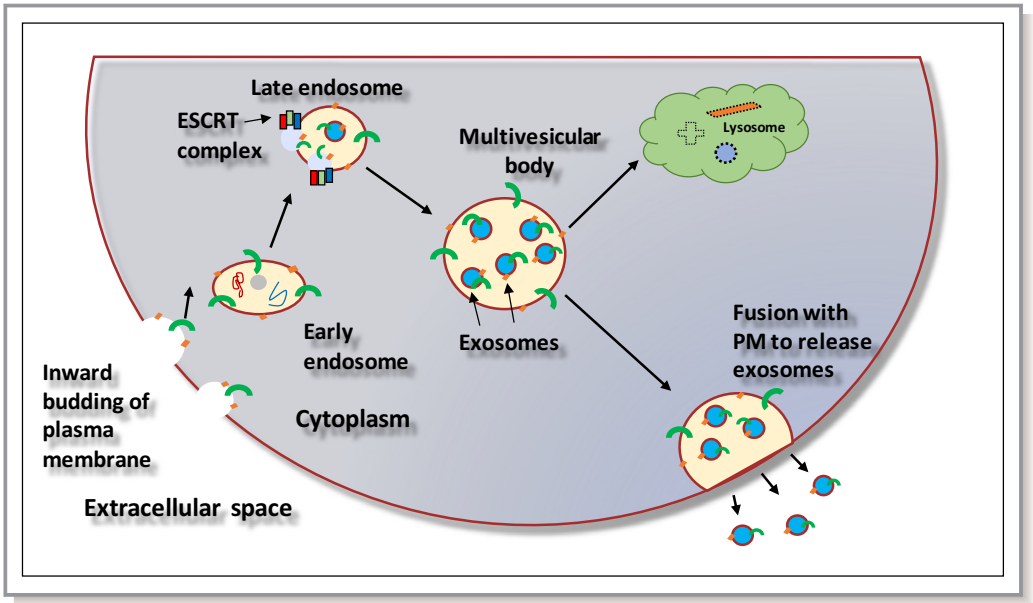

Figure 2. The formation and fates of multivesicular bodies. During endocytosis, the plasma membrane (PM) buds inward, filling with cytoplasmic contents and forming the early endosome. Surface proteins may be retained throughout endocytosis. The early endosome matures into the late endosome. This membrane further buds inward with the aid of the endosomal sorting complex required for transport (ESCRT), forming intraluminal vesicles. Once filled with these vesicles (now known as exosomes), the late endosome becomes the multivesicular body, which may either deliver its contents to the lysosome for degradation or fuse with the PM to secrete exosomes.

Isolation Techniques for Exosomes

To study exosome-mediated intercellular communication, exosomes need to be isolated and purified. A gold standard isolation method is lacking. Numerous isolation methods are available, but they result in an exosome populations with different yields, purity, and size.[58] In addition, proper preanalytical processing is important to minimize variability,[59] as the activation of cells during sample collection and handling has the potential to release additional exosomes. The isolation of exosomes from blood is especially problematic. The most common method of isolation is graded ultracentrifugation, and the exosomes can be purified further by the addition of a sucrose density gradient. Precipitation is also a common method that is commercially available through kits, although specificity is poor. Several studies in the literature examining the effects of exosomes may have used heterogeneous EV populations because of these limitations. Only size-exclusion chromatography results in the purification of a homozygous population. Lai et al used this method to purify a fraction of exosomes from MSCs.[18] These purified exosomes had a hydrodynamic radius of 55 to 65 nm and reduced infarct size when injected into an isolated heart model of ischemia and reperfusion injury. However, several studies which show EV-associated cardioprotection are presumably a mixed population of exosomes and/or microvesicles isolated from progenitor or stem cells.[60] This includes a study that showed MSC-conditioned medium containing both exosomes and

Table 1. Database Resources for Exosome Research

Name	Website Address	Information
Exocarta	http://www.exocarta.org/	Proteins, RNA, lipids
Vesiclepedia	http://www.microvesicles.org/	Proteins, RNA, lipids
EVPedia	http://student4.postech.ac.kr/evpedia2_xe/xe/	Proteins, RNA, lipids
EV Track	http://evtrack.org/	Database of EV publications
Plasma proteome database	http://www.plasmaproteomedatabase.org/	EV proteins from plasma

EV indicates extracellular vesicle.

microvesicles decreased infarct size following ischemic injury.[60] Consequently, it becomes less clear which homogenous population is critical when studying exosome-mediated intercellular communication.

Methods to Identify and Characterize Exosomes

The small size of exosomes makes their identification and characterization challenging; therefore, most studies use a combination of techniques to characterize the population of exosomes being studied. Lotvall et al described at set of minimum standards to meet for publication of scientific analyses involving exosomes and other vesicles.[61]

Nanoparticle tracking analysis using NanoSight (Malvern Instruments) provides a rapid method to characterize nanoparticles.[33] Particle size distribution and count is determined in real time using light scattering and Brownian motion of suspended samples. It allows the analysis of particles between 20 and 2000 nm and provides high-resolution particle size distribution profiles and concentration measurements. Despite the ease of use to characterize different samples, it gives the most accurate readings between 50 and 1000 nm; however, it is not specific to exosomes and counts every particle within its size range including membrane fragments and other cellular debris.[62]

Electron microscopy, with its superior resolution ability, is an important element of exosome characterization.[33] Exosomes are visualized by their characteristic negative staining with uranyl acetate and methylcellulose using transmission electron microscopy. They are identified as double-membrane "cup-shaped" vesicles that are also in the correct size range. The cup-shape description is most likely not their true form but rather an artifact of the procedure and should not be taken as a definitive feature of exosomes.[62,63]

For flow cytometry, the exosome size falls below the size threshold at which routine flow cytometers can accurately distinguish particles from noise.[64] This limitation is overcome by binding exosomes to latex beads either directly or indirectly through exosome-specific antibodies.[65] The bound exosomes can then be stained with other fluorophore-coupled antibodies. In this manner, surface proteins can be characterized, but the particle number may not be accurate because it is unknown how many exosomes bind to each bead.

The ability of exosomes to be taken up into cells can be determined by labeling the exosomes with lipophilic membrane-binding dyes such as PKH67, DiD, or CellMask.[66] These lipophilic dyes have also been shown to bind different types of lipoprotein particles such as low-density lipoprotein that are also taken up by the cell and thus may create artifacts. Consequently, it is important to purify exosomes to minimize contamination with lipoproteins and to use the appropriate controls to assess for artifactual uptake.[66] For certain studies, these issues can be overcome through labeling of specific vesicles by transfecting the parent cells with a vesicle-targeted fluorescent probe or reporter protein.[67]

Biological Roles for Exosomes in Cardiovascular Disease and Repair

Several studies suggest the role of exosomes as potential disease biomarkers. In one study, an exosomal miR was detected before any elevated levels of traditional disease biomarkers such as creatine kinase or troponin, highlighting the exosomes' biomarker potential for an early, sensitive, and specific diagnostic tool for cardiomyopathy.[68] Additional investigations have been exploring the use of exosomes as a delivery vehicle for various therapies,[69,70] given their ability to easily cross biological membranes.[71] Importantly, exosomes are being examined for their roles in various cardiomyopathies such as hypertrophy,[72,73] heart failure,[74] myocardial infarction, and ischemic injury.[68,75–77] These studies are discussed in more detail later and summarized in Table 2. It should be noted that the limitations of these studies include (1) inconsistent methods of exosome isolation that may lead to a mixed population of EVs being tested and (2) most of the mechanistic effects of exosomes being studied in in vitro models.

Cardioprotective Effects of Progenitor and Stem Cell–Derived Exosomes

Several studies examining the roles of CPCs, cardiospheres, and MSC-secreted exosomes have found their involvement in cardioprotective paracrine effects.[87] CPC-derived EVs, in which exosomes were a major component, decreased apoptosis in HL-1 cardiomyocytes 48 hours after serum starvation. The EVs were enriched in miR-132 and miR-210 and transferred these miRs to the cells; this was in part responsible for the antiapoptotic effects of the exosomes.[78] Furthermore, CPC-derived EVs increased myocardial viability, decreased cardiomyocyte apoptosis, and increased blood vessel density when injected into the myocardium in vivo.[78] The same effects were not seen using control exosomes isolated from dermal fibroblasts. Oxidative stress upregulates miR-21 within the exosomes isolated from CPCs,[79] and the transfer of exosomal miR-21 to H9C2 myoblasts decreases oxidative stress-induced apoptosis in vitro.[79]

Cardiospheres are multicellular clusters that contain progenitors of cardiomyocytes, endothelial cells, and smooth muscle cells.[88] Exosomes derived from cardiospheres have shown cardioprotective benefits in vitro by enhancing angiogenesis and cardiomyocyte proliferation and decreasing

Table 2. Study Summary

Study	Exosome Source	Exosome Prep	In Vitro Effects	In Vivo Effects
Barile 2014[78]	Isolated CPCs	1. ExoQuick precipitation solution 2. ultracentrifugation at 100 000*g* for 90 min 3. Column precipitation using Exo-spin kits	Decreases apoptosis in HL-1 cells	Improved cardiac function and angiogenesis
Xiao et al 2016[79]	Sca-1+ CPCs +/− H_2O_2	ExoQuick TC (System Biosciences)	H_2O_2–exosomes: Decrease oxidative stress in H9C2 myoblasts through miR-21 transfer	. . .
Ibrahim et al 2014[76]	Cardiospheres	ExoQuick TC (System Biosciences) 3 sizes of exosomes	1. Increase tube length of HUVECs 2. Increase proliferation of Ki67+ neonatal rat cardiomyocytes 3. Decrease apoptosis of neonatal rat cardiomyocytes	1. Decrease scar mass after myocardial infarct 2. Increase vessel density 3. Transfer of miR-146a
Aminzadeh et al 2018[80]	Cardiospheres	. . .	. . .	Exosomes injected in the heart improve skeletal muscle function.
Sahoo et al 2011[81]	MSCs	Ultracentrifugation	Increases HUVEC tube length, viability, and proliferation	
Yu et al 2015[82]	MSCs	ExoQuick Kit	Increase survival of cardiomyocytes exposed to hypoxia by decreasing apoptosis and preserving the mitochondria membrane potential	1. Intramyocardial injection into the peri-infarct region preserves cardiac function and decreases infarct size. 2. Transfer of miR-19a and miR-451 into the myocardium
Ribeiro-Rodrigues 2017[83]	H9C2 myoblasts +/− ischemia mimic conditions	Ultracentrifugation and sucrose gradient	Ischemia-derived exosomes: 1. Increase endothelial permeability 2. Increase EC proliferation and survival 3. Increase tube formation and migration	Ischemia-derived exosomes: 1. Improve ejection fraction after MI 2. Increase capillary density
Wang et al 2014[84]	Diabetic GK rat cardiomyocytes	Ultracentrifugation ExoQuick Kit Transwell coculture	Inhibit proliferation, migration, and tube formation of cardiac ECs by transfer of miR-320	
Davidson et al 2018[85]	1. Plasma from DM and non-DM patients 2. Nondiabetic and Goto Kakizaki type II diabetic rats 3. HUVECs +/− hyperglycemic conditions	Ultracentrifugation	. . .	. . .
Vicencio et al 2015[75]	Plasma from rats and healthy humans	Ultracentrifugation	HL-1 cells increase in mitochondrial membrane potential and cell death after hypoxia and reoxygenation	Decreased infarct size in a rat subject to ischemia–reperfusion injury

Continued

Table 2. Continued

Study	Exosome Source	Exosome Prep	In Vitro Effects	In Vivo Effects
Pironti et al 2015[13]	1. HEK293T cells overexpressing AT1Rs subject to osmotic stretch. 2. TAC/pressure overload serum exosomes 3. Cardiomyocytes are the source of these exosomes	Ultracentrifugation	1. Exosomes enriched for ATIRs 2. ATIRs transfer from exosomes to cardiomyocytes and skeletal myocytes	ATIR enriched exosomes confer blood pressure responses to angiotensin II in AT1R knockout mice
Bang et al 2014[72]	Neonatal rat cardiac fibroblasts	Ultracentrifugation Transwell direct-transfer experiments	Induces hypertrophy of neonatal cardiomyocytes	. . .
Datta et al 2017[86]	Hypertrophied cardiomyocytes	Ultracentrifugation	Modulates STAT-3 signaling in cardiac fibroblasts	. . .
Loyer et al 2018[10]	Heart tissues Myoectomy tissue from humans	Centrifuges at 20 500*g* to pellet large EVs and then exosomes isolated from Exosome Isolation Kit (Exiquon) to isolate small EVs	Used to stimulate cardiac-derived monocytes	Local generation of EVs in the heart following MI
Bian et al 2014[77]	Hypoxic MSC in vitro	Ultracentrifugation (100 000*g*); frozen (mixture of exosomes and MVs (47–180 nm)	HUVECs for proliferation, migration, and tube formation	Promotes angiogenesis and preserves cardiac performance in an acute MI model
Garcia et al 2016[7]	Cultured neonatal murine cardiomyocytes and H9C2 cells +/− glucose starvation	Transwell direct transfer experiments	Exosomes contain glucose transporters and transfer the functional transporters to HUVECs and primary ECs	
Hu et al 2018[11]	Cardiomyocytes	1. Differential ultracentrifugation 2. Rab27a knockout	Exosomes from injured cardiomyocytes increase BMSC apoptosis	Exosomes from injured cardiomyocytes accelerate BMSC injury

AT1R indicates angiotensin II type 1 receptor; BMSC, bone marrow–derived mesenchymal stem cell; CPC, cardiac progenitor cell; DM, diabetes mellitus; EC, endothelial cell; EV, extracellular vesicle; HEK293T, human embryonic kidney 293 cell line containing the SV40 large T antigen; HUVEC, human umbilical vein endothelial cell; MI, myocardial infarction; miR, microRNA; MSC, mesenchymal stem cells; MV, microvesicles; Sca-1, stem cells antigen-1; STAT3, signal transducer and activator of transcription 3; TAC, transverse aortic constriction.

apoptosis.[76] Cardiosphere-derived exosomes decreased cardiac infarct size in vivo via the delivery of miR-146a[76] and stimulated protective antioxidant and prosurvival signaling pathways.[80] Furthermore, Aminzadeh et al demonstrated that cardiosphere exosomes are capable of exerting crosstalk among multiple cell types; exosomes injected in the heart led to improved skeletal muscle function in mdx mice, a mouse model of Duchenne muscular dystrophy.[80]

MSCs originate in the bone marrow, and MSC-derived exosomes influence both endothelial cells and cardiomyocytes. MSC-derived exosomes modulate endothelial cells by stimulating angiogenesis and increasing viability, proliferation, and tube formation[81] and modulate cardiomyocytes by decreasing apoptosis and preserving mitochondrial membrane potential through the enrichment of miR-19a.[82]

Understanding the Paracrine Effects Exerted by Exosomes Secreted From Cardiac Cells in Cardiovascular Disease

Proper cardiovascular function depends on the coordinated interplay and communication between smooth muscle cells, endothelial cells, fibroblasts, immune cells, and cardiomyocytes. Exosomes contribute to this intracellular communication.[5,6] Despite exosomes representing an important mechanism for intracellular communication in general, little is known about the exosome regulation of cardiomyocytes and other noncardiomyocyte cell types within the healthy and diseased heart. Figure 3 summarizes current findings regarding the effects elicited by exosomes secreted from the different cardiac cell types in cardiovascular disease.

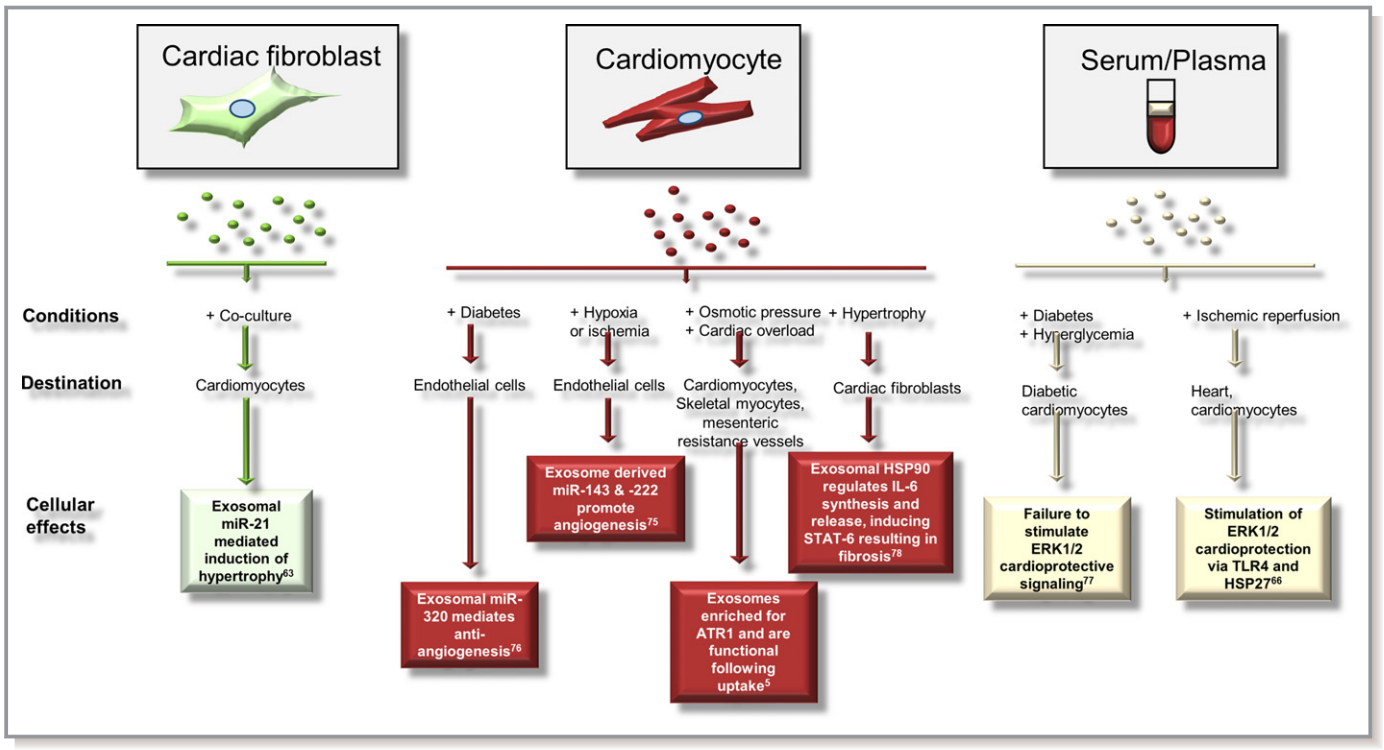

Figure 3. Paracrine effects in disease exerted by cardiovascular exosomes. Cardiovascular secreted exosomes exert diverse effects on their destination cells, considering the cell type of origin, exosomal contents, and the conditions of release. Cardiac fibroblast–derived exosomes have been associated with pathology because of their transference of microRNA (miR) 21, which led to the induction of cardiac hypertrophy.[70] The effects of cardiomyocyte-derived exosomes were either proangiogenic[82] or antiangiogenic,[89] dependent on the conditions of release, which affected their miR cargo. Cardiomyocyte-secreted exosomes were also found to be involved in the transfer of functional angiotensin II type 1 receptors (AT1Rs) under conditions of stress[12] and involved in HSP90 (heat shock 90-kDa protein)–dependent regulation of collagen synthesis in fibroblasts in models of hypertrophy.[83] Serum and plasma exosomes arise from a variety of cell sources, and the diversity of their effects results from the disease status and conditions of release. Diabetic serum exosomes fail to stimulate ERK1/2-protective signaling in cardiomyocytes,[90] whereas plasma exosomes from healthy controls could stimulate ERK1/2 (Extracellular signal-regulated kinase 1 and 2) via TLR4 (Toll-like receptor 4) and HSP27.[73]

Several studies suggest exosomes may change composition under pathological conditions. Recent studies have shown that miR profiles differ in plasma exosomes from spontaneously hypertensive rats compared with the normotensive Wistar–Kyoto rats.[89] Likewise, the protein content of exosomes differ among Zucker lean, Zucker fatty, and Sprague–Dawley rats.[90] In myocardial infarction, in which the heart is subject not only to hypoxic conditions but also to inflammation, cardiomyocytes increase the secretion of exosomes and change the composition of their cargo. H9C2 myoblasts subject to hypoxic conditions secrete exosomes enriched for miRs related to cell proliferation and differentiation including miR-143 and miR-222. In corollary experiments, the exosomes derived from the hypoxic cells induced endothelial proliferation, migration, and survival under stress.[83]

Exosomes do not always impart favorable effects on recipient cells and may be neutral to detrimental in exerting their effects. Exosomes released from diabetic GK rat cardiomyocytes inhibit the proliferation, migration, and tube formation of endothelial cell lines by a mechanism involving transfer of exosomal-associated miR-320.[84] Furthermore, diabetic conditions render exosomes ineffective in activating survival pathways in cardiomyocytes,[85] whereas in the absence of diabetes mellitus, cardioprotective effects were seen to activate the TLR-4 (Toll-like receptor 4)–ERK1/2 (Extracellular signal-regulated kinase 1 and 2) pathway.[75] HEK293T (human embryonic kidney 293 cells containing the SV40 large T antigen) cells overexpressing angiotensin II type 1 receptors (AT1Rs), when subject to osmotic stretch in vitro, secrete exosomes enriched for AT1Rs.[13] Likewise, in mice subject to transverse aortic constriction to induced pressure overload, the exosomes released into the serum had a 100-fold increase in AT1R density.[13] These AT1R-enriched exosomes transferred the functional AT1R to recipient wild-type HEK293 cells in vitro and to the heart and skeletal muscle of AT1R-knockout mice in vivo.[13] Exosomes have also been shown to mediate cross-talk between cardiomyocytes and cardiac fibroblasts in cardiac hypertrophy. Cardiac fibroblast–derived exosomes enriched in miR-21-3p have been shown to be

potential regulators of cardiomyocyte hypertrophy.[72] Likewise, exosomes secreted from hypertrophied cardiomyocytes contain HSP90 and IL-6 (interleukin 6) to maintain STAT3 (signal transducer and activator of transcription 3)–fibrotic pathway signaling.[86] This study, however, also showed differential regulation of collagen expression in the cardiac fibroblast in vitro versus in vivo, which points toward the potential paracrine effects resulting from crosstalk among different cell types. It will be important for future studies to compare in vivo findings with in vitro systems to confirm which cell type is releasing exosomes to exert specific effects.

It has recently been shown that exosomes derived from injured cardiomyocytes may impede the survival of bone marrow–derived MSCs in the infarcted heart.[11] Exosomes isolated from cultured cardiomyocytes treated with H_2O_2 could induce bone marrow–derived MSCs to undergo apoptosis in vitro. In corollary in vivo experiments, Ras-Related Protein Rab27a-knockout mice were engineered to have reduced exosome release from cardiac tissues. After left anterior descending artery ligation in the Rab27a-knockout mouse heart, mouse bone marrow–derived MSCs were injected into the border zone of the infarct. These transplanted bone marrow–derived MSCs showed 3-fold increased survival after 4 days.[11] The accumulative evidence suggests that EVs, including exosomes, when produced by cells under adverse conditions, not only contribute to the disease pathology but also may be detrimental toward cardiac regenerative therapies. Nevertheless, the full extent of EV-mediated intracellular communication within the heart and how neighboring or distant cells can secrete EVs to influence the cardiac structure and function are largely unknown. The Rab27a-knockout mouse and the continued development of cell-type promoter CD63–GFP (green fluorescent protein) transgenic animals[91,92] are steps toward addressing these knowledge gaps.

iPSCs as a Tool to Understand Intercellular Communication in Disease

The extracellular spaces of the myocardium contain exosomes from a mixed cellular source, making it difficult to determine the influence of cell-type–specific exosomes from one cell on its neighboring cell. Consequently, in vitro studies are critical to understanding specific cell-type exosome function in health and disease. An important consideration in the interpretation of all exosome studies is the type of model system used in the investigation. In vivo, there is likely to be a mixture of EVs from multiple cell types present in the extracellular space, whereas in vitro, only cell-type–specific EVs can be examined individually. Furthermore, the addition of exogenous exosomes (in vitro or in vivo) may not be physiologically accurate, which is critical in the interpretation of results.

iPSCs as disease models can be used to model intercellular communication between neighboring cell types in the heart by studying the effects of cell-type–specific exosomes. Numerous studies have validated the use of iPSCs as a model system to study genetic cardiac diseases such as long QT syndrome,[27,93] LEOPARD syndrome,[94] and Duchenne muscular dystrophy.[29,95] In addition, there is growing interest in using iPSC-derived cells to study environmental modifiers of disease. The iPSC-derived cells can be exposed to various conditions to precipitate oxidative, metabolic, or endoplasmic reticular stress or subjected to neurohormones, growth factors, or hypoxia to mimic certain disease states. For example, a diabetic environment can be created by supplementing the culture media with glucose, endothelin 1, and cortisol.[32]

Exosomes are also modulators of the disease process. The relevant recipient cell of interest can be cardiomyocytes, endothelial cells, smooth muscle cells, or cardiac fibroblasts. Exosomes can be isolated from one cell type and be applied to another cell type to assess phenotypic changes. For instance, iPSC-derived endothelial cells exposed to exosomes isolated from iPSC-derived cardiomyocytes can be assessed for changes in gene expression and various phenotypes such as tube formation, wound healing, senescence, and low-density lipoprotein uptake (Figure 4). The contributions of different genetic backgrounds or gene mutations on exosome cargo and the resulting phenotypic effects of exosomes on recipient iPSC-derived cells can also be studied using patient-specific iPSCs. Furthermore, 3-dimensional organoid models have been developed to investigate EV-mediated intercellular communication.[67] This model is based on coculturing recipient cells with donor cells containing a CD63–GFP reporter under control of a cell-type–specific promoter. In this manner, EV-mediated intercellular communication can be investigated under conditions closer to the physiological environment.

Exosome cargo can be compared from wild-type and diseased iPSC lines and derived cells. Our laboratory and others have validated the use of iPSC-derived cell lines as a model for cardiovascular disease[29,95] and showed that exosomes derived from these cell lines may play a role in the disease process related to their cargo. The miRs elicit functional effects in cells typically by inhibiting targeted gene expression. Dysregulation of miRs is associated with dilated cardiomyopathy.[96] In Figure 5, we show that miR cargo differs not only between wild-type and Duchenne muscular dystrophy iPSC-derived cardiomyocytes but also between Duchenne muscular dystrophy iPSC-derived cardiomyocytes and skeletal myocytes. Furthermore, cell-type–specific exosome cargo can be compared under basal conditions along with different types of stress conditions such as hypoxic, metabolic, oxidative, and hyperglycemic stresses. Malik et al showed that pathologic changes to the environment such as hypoxia and ethanol

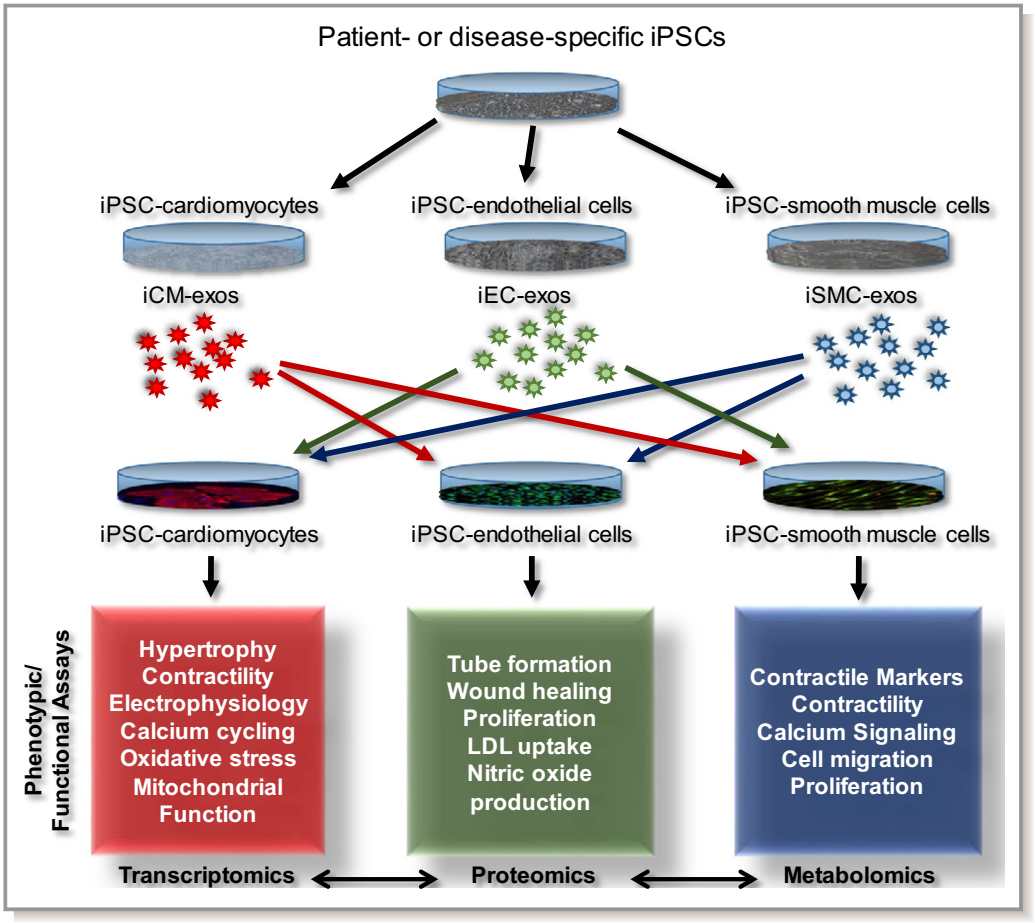

Figure 4. Using induced pluripotent stem cells (iPSCs) to study exosomal communication between cardiovascular cell types. Patient- or disease-specific iPSCs can be differentiated into multiple cell types including cardiomyocytes, endothelial cells, or smooth muscle cells. These derived cells can be used to collect and profile exosomes (exos) or to assess cell-type–specific exosomal effects on recipient cell function. iCM indicates iPSC-derived cardiomyocyte; iEC, iPSC-derived endothelial cell; iSMC, iPSC-derived smooth muscle cell; LDL, low-density lipoprotein.

change protein content and reactive oxygen species decrease exosome production from cardiac myocytes.[44]

The iPSC-derived cells can also be used to decipher the function of exosomes compared with other EVs. Experiments using GW4869, a specific noncompetitive inhibitor of neutral spingomyelinase, is commonly used to inhibit the secretion of exosomes; however, it blocks the secretion of EVs in general. Consequently, the in vitro and in vivo findings with the compound can be conflicting. For instance, serum EVs isolated from the Duchenne muscular dystrophy (mdx) mice decrease apoptosis in C2C12 myocytes; however, mdx mice treated with GW4869 show improved muscle strength in vivo.[97] In addition, GW4869 has been shown to exert cardioprotective effects against sepsis-induced cardiac dysfunction.[98] Others have found cardioprotection with the addition of serum EVs.[75,99] A consideration for the interpretation of results with the use of serum EVs and their global blockade with inhibitors is that serum EVs contain exosomes, microvesicles, and apoptotic bodies, all originating from a variety of cellular sources, so it can be difficult to pinpoint which vesicle type and source is responsible for the effects observed without purifying the EV subtype of interest and doing isolated cell studies. Because stimuli and originating cell type affect the contents of exosomes, it is likely that the same cell type can release both protective and pathogenic exosomes depending on its status at the time of release.

Conclusions

Exosomes play an important role in the function of the heart under normal and disease conditions. Whereas exosomes harvested from stem cells in vitro may protect against cardiac injury and promote repair, the exosomes secreted from the endogenous cardiac cells may play a more ominous role in cardiac disease progression. However, we are just beginning to understand how the exosomal cargo changes under different

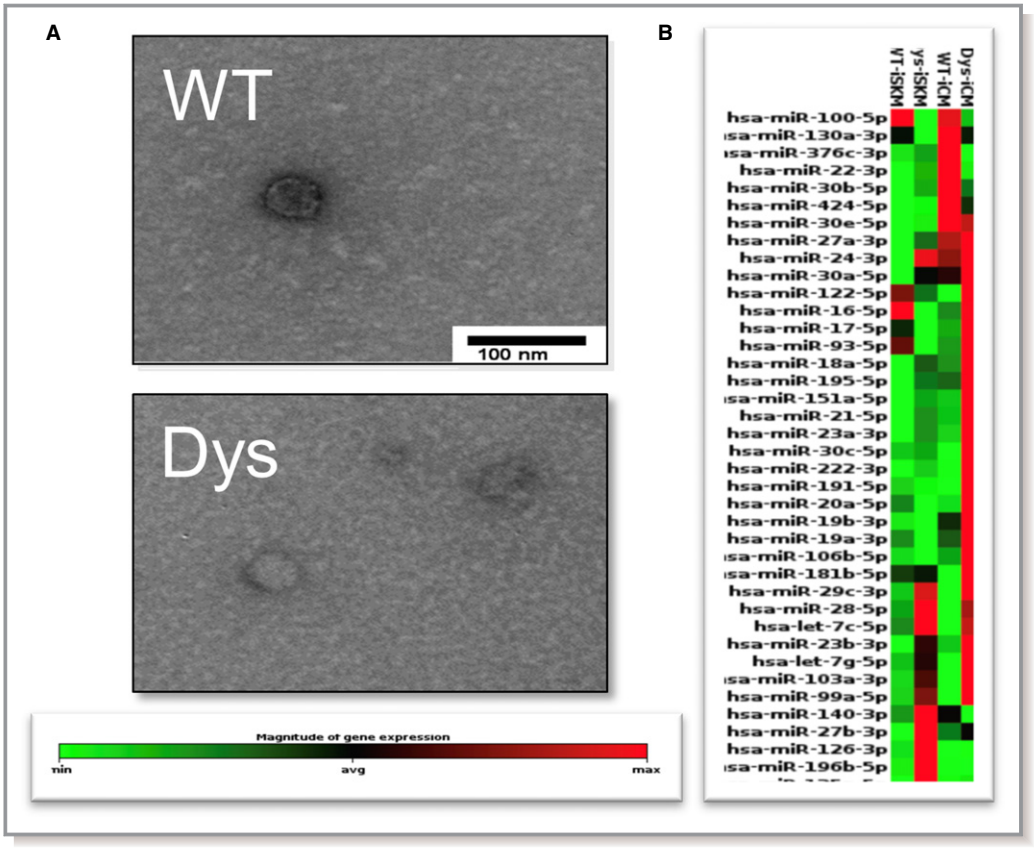

Figure 5. Example of using induced pluripotent stem cell (iPSC)–derived cells to investigate cell- or disease-specific exosome cargo. Exosomes were isolated from wild-type (WT) and dystrophin-deficient (Dys)-iPSC-derived cardiac and skeletal myocyte-conditioned media using an isolation reagent by Thermo Fisher. A, Isolated exosome size (50 nm) and morphology was confirmed by electron microscopy. B, WT and Dys cardiac and skeletal myocyte exosomes display differential microRNA (miR) profiles, as shown by polymerase chain reaction array analysis. hsa indicates homo sapiens; iCM, iPSC-derived cardiomyocytes; iSkM, iPSC-derived skeletal muscle cells; let, part of the lethal-7 gene family.

conditions and how this affects the signaling pathways and phenotypic behavior of recipient cells. More studies are needed to illuminate the mechanisms that influence the sorting of exosomes and the diverse paracrine effects exosomes have in cardiovascular disease processes. Harnessing the potential of the translational power of exosomes by understanding the fundamental roles of exosomes in cardiovascular disease will provide insights for the discovery of novel disease mechanisms and the development of novel therapeutics.

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Disclosures

None.

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