Minireview

Therapeutic potentials of stem cell–derived exosomes in cardiovascular diseases

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Impact Statement

Stem cell–derived exosomes have been shown to regulate a variety of stresses, either inhibiting or promoting cell balance. Exosome-based cell-free therapies have been evaluated for several disorders by exploiting the paracrine activity of mesenchymal stem cell (MSC)-derived exosomes. This review highlights the bioengineering of fabricated exosomal cargoes, which could shed light on the role of exosomes and provides a new avenue for the treatment of cardiovascular diseases.

Abstract

Exosomes are extracellular vesicles released by many cell types with varying compositions. Major bioactive factors present in exosomes are protein, lipid, mRNA, and miRNA. Exosomes are fundamental regulators of cellular trafficking and signaling in both physiological and pathological conditions. Various conditions such as oxidative stress, endoplasmic reticulum stress, ribosomal stress, and thermal stress alter the concentration of exosomal mRNA, and miRNA, lipids, and proteins. Stem cell–derived exosomes have been shown to regulate a variety of stresses, either inhibiting or promoting cell balance. Stem cell–derived exosomes direct the crosstalk between various cell types which helps recovery by transferring information in proteins, lipids, and so on. This is one of the reasons why exosomes are used as biomarkers for a multitude of disease conditions. This review highlights the bioengineering of fabricated exosomal cargoes. It includes the manipulation and delivery of specific exosomal cargoes such as noncoding RNAs, recombinant proteins, immune modulators, therapeutic drugs, and small molecules. Such therapeutic approaches may precisely deliver the therapeutic drugs at the target site in the management of various disease conditions. Importantly, we have focused on the therapeutic applications of stem cell–derived exosomes in cardiovascular disease conditions such as myocardial infarction, ischemic heart disease, cardiomyopathy, heart failure, sepsis, and cardiac fibrosis. Generally, two approaches are being followed by researchers for exosomal bioengineering. This literature review will shed light on the role of stem cell–derived exosomes in stress balance and provides a new avenue for the treatment of cardiovascular diseases.

Keywords: Stem cell–derived exosomes, miRNA, cardiac diseases, bioengineering, drug delivery

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Introduction

Cardiovascular diseases (CVDs) have plagued human society over a long period, impacting the quality of life and mortality rate in the patient population and creating economic havoc.[1] In the milieu of several CVDs, cellular degeneration and dysfunction are one of the primary causes, leading to cumulative organ damage and decline in its function. CVDs are one of the co-morbidities of aging-associated degenerative conditions.[2] Regenerative medicine offers a new prospect for developing therapeutics for degenerative diseases including CVD.

One of the facets of regenerative medicine are stem cells, which are a subset of heterogeneous cells that can differentiate into specialized cell types as well as have the ability to self-renew. Based on their origin, stem cells can be classified into (1) embryonic stem cells obtained from the inner cell mass (iCM) of the blastocyst and (2) adult stem cells obtained from adult tissues such as bone marrow, adipose tissue, and so on.[3] Another classification of stem cells is based on their ability to differentiate (1) totipotent stem cells, which can form an entire organism (e.g. zygote); (2) pluripotent stem cells, which can form all the cell types of three germ layers such as ectoderm, mesoderm, and endoderm (e.g. embryonic stem cells); (3) multipotent stem cells, which can be differentiated into many cell types (e.g. mesenchymal stem cells [MSCs], hematopoietic stem cells [HSCs], bone

marrow, adipose-derived stem cells); (4) oligopotent stem cells, which can be differentiated into a narrower range of cell types than multipotent cells (e.g. myeloid stem cells); and (5) unipotent stem cells, which can be differentiated into one specific cell type (e.g. osteocytes).[4,5] Of these, induced pluripotent stem cells (iPSCs),[6] MSCs,[7] skeletal myoblasts,[8] cardiac stem cells,[9] HSCs,[10] and endothelial progenitor stem cells[11] have been widely considered as potential candidates for cardiovascular therapeutics.

MSCs are an umbrella term used to describe a diverse population of multipotent stem/stromal cells that are considered as a crucial constituent to the field of tissue engineering. These cells are readily isolated from bone marrow and adipose tissues. But MSCs are ubiquitous and are present in almost all tissues as a part of their structure. There are certain striking properties specific to MSCs including proliferation into colonies (measured in colony forming units-fibroblasts [CFU-F]), high nucleus:cytoplasm ratio,[12] and trilineage differentiation, that is, under specific *in vitro* conditions, MSCs can differentiate into endoderm (alveolar epithelial cells), ectoderm (neural cells), and mesoderm (cardiomyocytes) cell types.[13] MSCs have comprehensive therapeutic values as they exhibit immunomodulatory and anti-inflammatory properties by secretion of cell-surface growth factors such as vascular endothelial growth factor, granulocyte-colony stimulating factor, macrophage colony stimulating factor, insulin-like growth factor-1, and cytokines such as TGF-β, TSG-6, iNOS, IL-1, IL-6, IL-7, and IL-8. These cells also play a part in macrophage polarization from pro-inflammatory (M1) to anti-inflammatory macrophages (M2). The abovementioned factors work together in unison to efficiently regulate the immune system. Hence, MSCs are ideal candidates for allogeneic transplants.[12] MSCs delivered into the heart have played important roles in neovascularization, immunomodulation, and endogenous tissue repair, including reduction of fibrosis.[14–16] Numerous preclinical and clinical studies have evaluated the effects of MSCs on the repair and recovery of cardiac tissues. MSCs are effective in ischemic and non-ischemic heart failure by stimulating endogenous cardiomyocyte proliferation, improving left ventricular function, and reducing fibrosis.[17–21]

Exosomes are nanosized vesicles of 30–200 nm in diameter bound by a single membrane enriched in lipids, glycoconjugates, and proteins. They have a high molecular heterogeneity, expressing an assemblage of high-order membrane-associated proteins, nucleic acids (DNA, RNA), and lipids. They are mainly involved in paracrine signaling to target cells, carrying out important signaling processes, intracellular trafficking of biomolecules such as proteins, development of cancer,[22,23] protein quality control,[24,25] and cell membrane remodeling.[26,27] There has been a huge paradigm shift in MSC therapeutics, which relies on the ability of exosomes to act as intercellular messengers. Exosome-based cell-free therapies have been evaluated for several disorders by exploiting the paracrine activity of MSC-derived exosomes.[12] Since MSC-derived exosomes are widely used in clinical applications, this review has been focused on the exosome-based therapies, specifically in the context of CVD.

Sources of exosomes

At the beginning, scientists presumed exosomes were a waste management system to recycle or discard unwanted products, as it was widely observed in bodily fluids, including blood, ascites, cerebrospinal fluid, saliva, breast milk, tears, bronchial lavage, sweat, semen, and urine.[28,29] As they looked deeper into the function of exosomes, they realized its role in cell–cell signaling and metabolic pathways. Exosomes are usually secreted in culture by all cell types, including neural, adipocytes, cardiomyocytes, hematopoietic, epithelial, and cancer cells. But researchers have focused mostly on stem cell–derived exosomes for clinical applications. MSCs are an excellent source of cells for obtaining exosomes and are playing an important role in their immunomodulatory properties.[30] Exosomes from bone marrow stem cells have been known to delay osteoarthritis by promoting macrophage polarization to M2[31] and reducing osteoporosis through long non-coding RNA MALAT1.[32] They have also been used for the treatment of COVID-19 due to their ability to reduce cytokine storm and reinstate oxygenation.[33] Adipose stem cell–derived exosomes have been shown to promote wound healing via Wnt/β-catenin and PI3K/Akt pathway.[34] MSC-derived exosomes have also been shown to demonstrate cardiac wound healing through their anti-apoptotic effects via the transfer of miR-125b[35] and targeting pro-apoptotic proteins through exosomal miR-25-3p.[36] HSC-derived exosomes have been shown to express several pro-angiogenic, anti-apoptotic genes such as vascular endothelial growth factor, basic fibroblast growth factor (bFGF), IGF-1, and IL-8.[37] Exosomes derived from HSC enriched with miR-126 improved neovascularization after ischemia.[38] Exosomes derived from dendritic cells[39] and endothelial progenitor cells were able to improve neovascularization and cardiac function.[40] In particular, endothelial progenitor stem cells have been known to promote cardiac neovascularization through paracrine secretion of cytokines and growth factors.[40] Wharton's jelly MSC-derived exosomes secrete programmed death ligand 1 (PDL-1) and suppress T-cell, and thus, they avoid immunological rejection (graft versus host disease), a major problem in stem cell therapy.[41] They have also been shown to promote macrophage polarization, promote osteochondral regeneration, and enhance bone marrow-derived MSCs (BMSCs) and chondrocytes' proliferation and migration.[42] Furthermore, Wharton's jelly MSC-derived exosomes have been identified as a suitable candidate for drug delivery and tumor silencing through siRNA delivery.[43] Mouse embryonic stem cell–derived exosomes have been shown to reinstate heart function and modulate cardiomyocyte repair through combination therapy with cardiac progenitor cells (CPCs).[44] Recently, iPSCs and their subsequent differentiated derivates, iPSC-iMSC-derived exosomes, have been considered as a potential candidate for clinical trials due to their high plasticity and immunomodulatory properties. iPSC-derived iMSCs have been shown to possess higher proliferation, survival, and therapeutic capabilities in comparison to adult MSCs.[45] Not much progress has been made in this area as the underlying mechanism surrounding iPSCs has not been properly elucidated.

Structure and composition of exosomes

Exosomes are single membrane, circular nanovesicles that possess heterogeneous molecular topology. Just like a normal cell membrane, an exosomal membrane consists of a lipid bilayer made up of phospholipids (phosphatidylcholine, phosphatidylserine, phosphatidylinositol, ceramides, sphingomyelin),[46] glycoconjugates (heparinase, α-2,6-sialic acid, hyaluronan synthase 3),[47] and a comprehensive list of transmembrane proteins and lipid-anchored and peripherally associated membrane proteins.[48] Exosomal payload varies based on the parent cells that secrete them. According to the ExoCarta database (http://www.exocarta.org/), 1116 lipid species, 2838 MicroRNA (miRNA), 3408 mRNA, and 9769 species of proteins have been identified.[49] Membrane proteins that are abundantly present on exosomes include the tetraspanin family of proteins (CD63, CD81, CD9, CD37),[50] immunoglobulin superfamily 8 (IGSF8),[51] heat shock proteins (Hsp 90),[52] endosomal proteins (Alix), endosomal sorting complex required for transport (ESCRT 0, I, II, III), tumor suppressor gene 101 protein (TSG101),[53] gag retroviral proteins (Gag),[54] syndecans (SDC1-4),[55] milk fat globule protein E8 (MfgE8), prion proteins, and so on. Of these, the tetraspanin proteins, ESCRT, and Alix proteins have been shown to be involved in exosome biogenesis and widely used as biomarkers for exosome detection. It also contains a multitude of enzymes such as lipases, phosphatases, pyrophosphatases, proteases, and glycosyltransferases[26] and other key enzymes required for enzyme metabolism.[56] Furthermore, an important exosomal component involved in cell–cell signaling is RNA, particularly mRNA and miRNA.[57] miRNAs from tumor exosomes are widely used as biomarkers by oncologists due to their unique molecular signature.[58] Exosomes also contain DNA, including genomic DNA (gDNA), mitochondrial DNA (mtDNA), single-stranded DNA (ssDNA), and double-stranded DNA (dsDNA).[59] Although the components of the exosomes have been identified, the sorting machinery of the exosome cargo is largely unknown. Understanding how certain molecules are packaged into the exosomes is critical to analyze the exploratory pathways and how they affect the metabolism and cellular signaling in the target cells.

Exosome biogenesis

Exosome formation usually begins with the internalization of cargo from the plasma membrane through clathrin-dependent or independent (caveolin-mediated) endocytosis, macropinocytosis, phagocytosis, and lipid raft–mediated internalization with the help of SNARE, tetraspanins, and Hsp proteins.[48] This is followed by vesicle budding into the endosomal compartment where it mixes with cargo from the endoplasmic reticulum forming early endosomes. Here, the cargo is sorted into exosomes through the ESCRT proteins.[60] Upon maturation, late endosomes give rise to intraluminal vesicles to form multivesicular bodies, which then fuses with the plasma membrane to release exosomes into the extracellular space. However, not all exosomes are released. Some exosomes contain cellular waste that need to be degraded by directing them to lysosomes.[61] We do not know much about this bipartite deciding mechanism, but there is a report showing that exosomes associated with multivesicular bodies rich in cholesterol are released into the extracellular space, while those with cholesterol-poor membranes are prone to lysosomal degradation.[62] The two common modes of exosome release include direct vesicle release from the plasma membrane as seen in glioblastoma exosomes[63] and the role of arrestin domain-containing protein 1 in microvesicles. This has been identified for packaging and intracellular delivery of a myriad of macromolecules, including the tumor suppressor p53 protein, RNAs, and the genome-editing CRISPR-Cas9/guide RNA complex.[64] Although the idea of exosomal biogenesis through the endosomal compartment is widely accepted, that does not mean exosomes are generated only through this pathway. Another method of exosome biogenesis is intracellular plasma membrane–connected compartments (IPMCs), involving the delayed release of exosomes followed by the opening of the IPMC necks (Figure 1).[65] It is little surprising that only the endosomal mode of biogenesis has been widely accepted. It could be due to the observational bias since exosomes are not able to be identified from other modes as most of them get washed away during the centrifugation step, in particular plasma membrane exosomes. All of the electron microscopy images over-sample the endosomal mode. This observational bias can only be corrected by refocusing electron microscopy on other modes of biogenesis and performing a comparative microscopic analysis of both endosomal and IPMC modes of exosome biogenesis.[48]

Exosome uptake

There are multiple modes of exosome uptake, including phagocytosis, macropinocytosis, and clathrin-dependent and clathrin-independent endocytosis. Exosome uptake routes are varied, and it depends on the type of donor and recipient cells.[66] But mostly these pathways involve exosome internalization by the plasma membrane. Exosome uptake has been compared with viral entry. However, the one aspect of exosome uptake that varies with the viral entry is in its molecular heterogeneity, as exosomes have an array of heterogeneous transmembrane proteins.[48] On the contrary, similar to retrovirus, exosomes also evade degradation while entering the cell.[48] Exosome uptake also depends on the composition of the lipid bilayer from the plasma membranes of the recipient cells in the form of expression of cell adhesion molecules, integrins, phosphatidylserine, glycans, and other adhesion molecules.[67]

Bioengineering of exosomes

Exosomal bioengineering involves modifying exosomal cargo and membrane composition for easy uptake and producing a pronounced effect in the target cells. Due to their smaller size, structural similarity to the normal cell membrane, negative zeta potential, biocompatibility, and immune-evasive characteristics, exosomes are able to pass through natural barriers like the blood–brain barrier and thus are ideal candidates for bioengineering and drug delivery.[68] Despite their advantages, the main drawbacks on the clinical application of exosomes are in the isolation

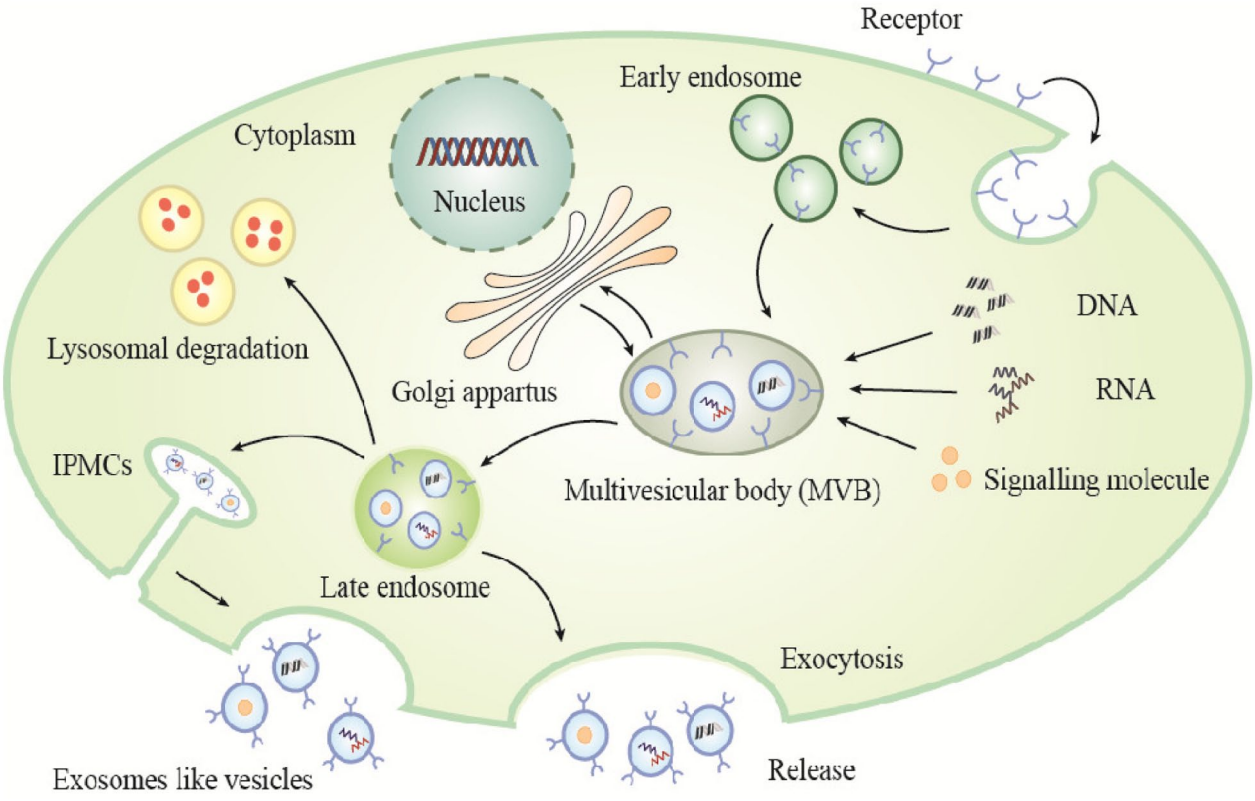

Figure 1. Exosome biogenesis and release: the formation of exosomes usually begins with the internalization of cargo from the plasma membrane. Internalized cargo enters the intracellular sorting organelles, known as endosomes. Here, the cargo (RNA, proteins, signaling molecules) is sorted into exosomes. The exosomes that arise from matured endosomes form multivesicular bodies (MVBs), which are then bifurcated, either for release or lysosomal degradation. The release of exosomes occurs by fusion of MVBs with plasma membrane. Another method of exosome release involves delayed release through budding at intracellular plasma membrane–connected compartments (IPMCs), followed by deconstruction of IPMC necks.

and large-scale production of them, systematic drug loading, and streamlined distribution and delivery to the target cells.[69] Generally, two approaches are being followed by researchers for exosomal bioengineering. A simple approach is that exosome-producing cells are incubated with the cargo (miRNA, siRNA, mRNA, or macromolecules like protein) or with inducer drug, small molecule followed by collection of modified exosomes. In the second approach, the exosomes are being collected from the culture medium or serum first, followed by treatment with different cargoes by incubation, electroporation, and sonication. This method has its drawbacks as it is laborious and requires proper physical and chemical conditions.[70]

Modifying the exosomal membrane involves coating of the membrane with chemical conjugates such as polyethylene glycol, a hydrophilic polymer to enhance the duration of circulation of the nanovesicle and enhance their specificity to target cells,[71] and with cholesterol, recombinant proteins, intercalating dyes,[72] and tannic acid for the enhanced binding of proteins to the myocardium.[73] Lipid bioconjugates composed of proteins such as streptavidin[74] and peptides[75] can also be anchored to the exosome membrane. Functional groups such as amines[76] and carboxylic acids[77] are present on the exosome membrane through a linker, so the peptide of our interest can be detected by a series of reactions involving click-chemistry.[78] Another approach is the genetic modification of the exosome-secreting cell to express the peptide of our interest, ultimately leading to the incorporation into exosomes as well.[79] One such example is the bioengineering of cardiospheres to express lysosome-associated membrane protein 2 (LAMP2B), an exosomal membrane protein that has been shown to increase cardiac retention in mice.[79] Exosome bioengineering can also be performed to modulate exosome trafficking and decide the fate of exosomes. One strategy is to disrupt the endolysosomal membrane through the fusion of exosomes with pH-sensitive peptides and cationic lipids for efficient cytosol release.[80] Another approach is to stimulate micropinocytosis by utilizing arginine-rich cell-penetrating peptides for a higher yield of cytosolic exosomes.[81] Surface modifications can also be done for the detection and tracking of exosomes by incorporating a fluorophore, radiolabeled proteins, and recombinant proteins.[72] Surface engineering of exosomes with aptamers, ligands, and antibodies is another direction that can be used only for a particular subset of target cells. This aptamer approach utilizing SELEX technology[82] involves surface modification of tetraspanin proteins and can be used as nanoprobes for the early detection of cancer. This method is rapid and highly sensitive, so the detection of disease or cancer cells could also be efficient.[83]

The second stratagem for exosome bioengineering involves modulating the culture, physical, and chemical conditions. One approach is to culture cells in stress-inducing conditions such as hypoxia,[84] serum starvation,[84] and

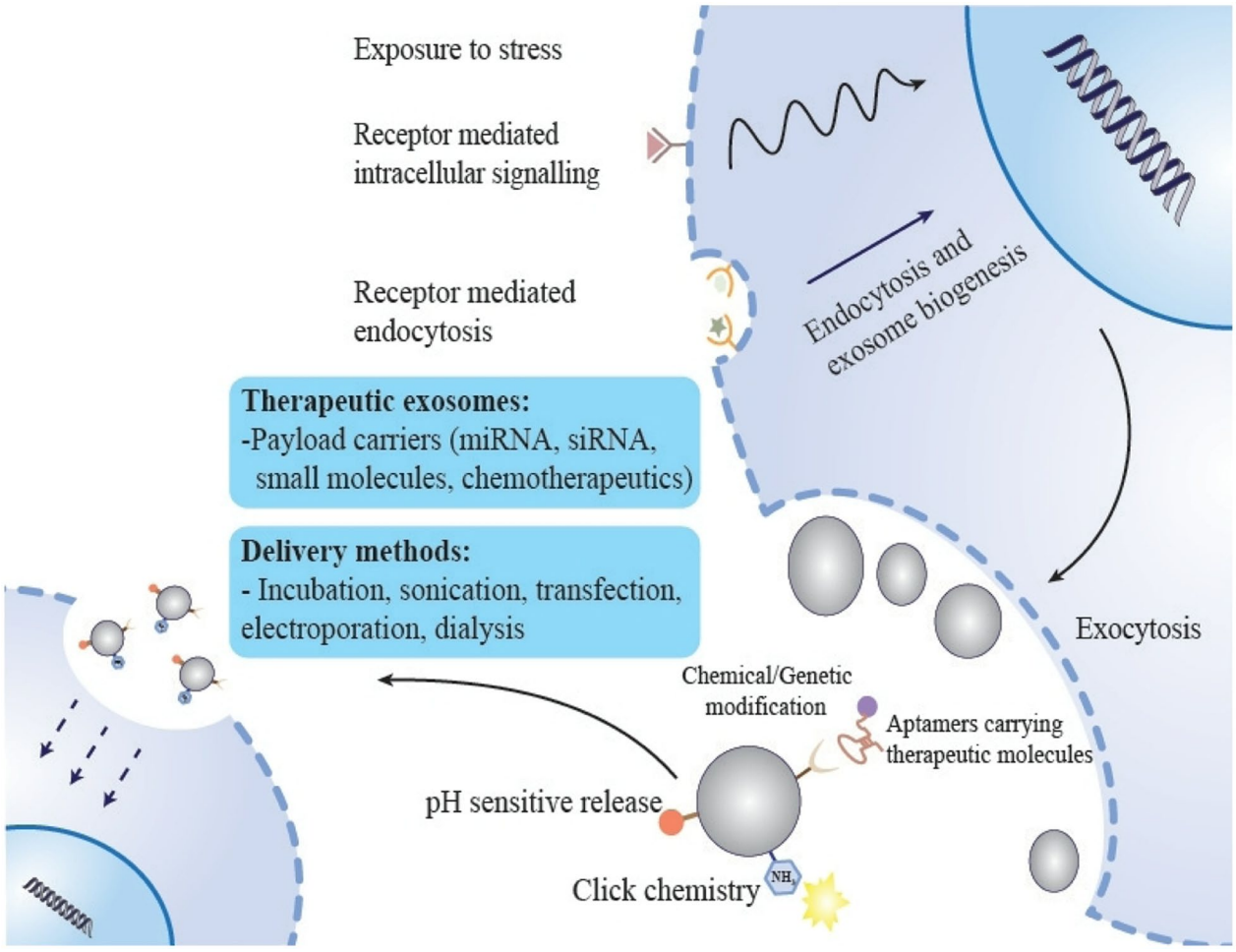

Figure 2. Bioengineering of exosomes. Exosomes can be bioengineered to modify exosomal cargo and membrane composition for easy uptake and producing a desired effect in the target cells. It involves two techniques, (1) modifying exosome membrane using chemicals and (2) modifying the culture conditions. Surface engineering of exosomes using lipids and cations is performed for enhanced specificity and detection. Cells exposed to various stresses, such as heat, hypoxia, serum starvation, and inflammation, are known to modify the content of the exosomes via receptor-mediated endocytosis or receptor-mediated intracellular signaling. In addition, exosomes can be used as a biological carrier of therapeutic miRNAs, siRNAs, aptamers, or small molecules for the targeted delivery into tissues. Exosomes are then delivered to the recipient cell through electroporation and incubation.

inflammation.[85] This leads to genetic reprogramming of the cells to modify exosomal cargo to carry genes required for alleviating a particular condition. When exposed to hypoxic conditions, CPCs can secrete exosomes containing miRNAs such as miRNA-15b, miRNA-17, miRNA-20a, and miRNA-103, which cause enhanced tube formation, reduced TGF-β expression, and mitigation of tissue damage.[86] This idea can also be incorporated with the transfection of miRNAs, siRNAs, and mRNA to increase the specificity of the target. Cardiac exosomes–derived miRNAs have been shown to produce various effects, for example, (1) miR-1 alleviated oxidative stress, inhibited cardiac hypertrophy, and induced cardiac-specific differentiation; (2) miR-133a inhibited fibrosis, and promoted cardiomyocyte survival and differentiation; and (3) miR-208a/b promoted cell growth and upregulated sarcomeric gene expression.[87–90] Empowering exosomes with non-coding RNA help in regulating several pathways and plays an important role in tumor metastasis through regulation of niche. Short interfering RNA (siRNA) used in RNA interference (RNAi) can also be delivered by exosomes. This exosome-mediated delivery of siRNA to the mouse brain was achieved after systemic injection of targeted exosomes.[91]

Hybrid approaches involving adeno-associated viruses (AAV)-encapsulated exosomes have been implemented since exosomes and viruses have similar biogenesis, making them resistant to AAV-neutralizing antibodies with increased efficiency.[92,93] This approach can also be used in suppressing viral infection since exosomes secreted by viruses code for viral proteins, making them identical to a non-infectious virus (Figure 2).[68,94] So far, several methods of engineered exosomes have been developed and have been applied to *in vitro* and *in vivo* cultures. But, only a few of them have reached the stages of clinical trials. This is due to a lack of proper demonstration of the efficacy, safety, and utility of these exosomes. Most importantly, large-scale production of exosomes in obedience to good manufacturing practices (GMPs) is still called into question. To circumvent these drawbacks, computational methods have been applied and stimulations are being carried out for high-throughput analysis in clinical settings. More research should be focused to enhance the viability of exosomes in clinical applications through bioengineering.

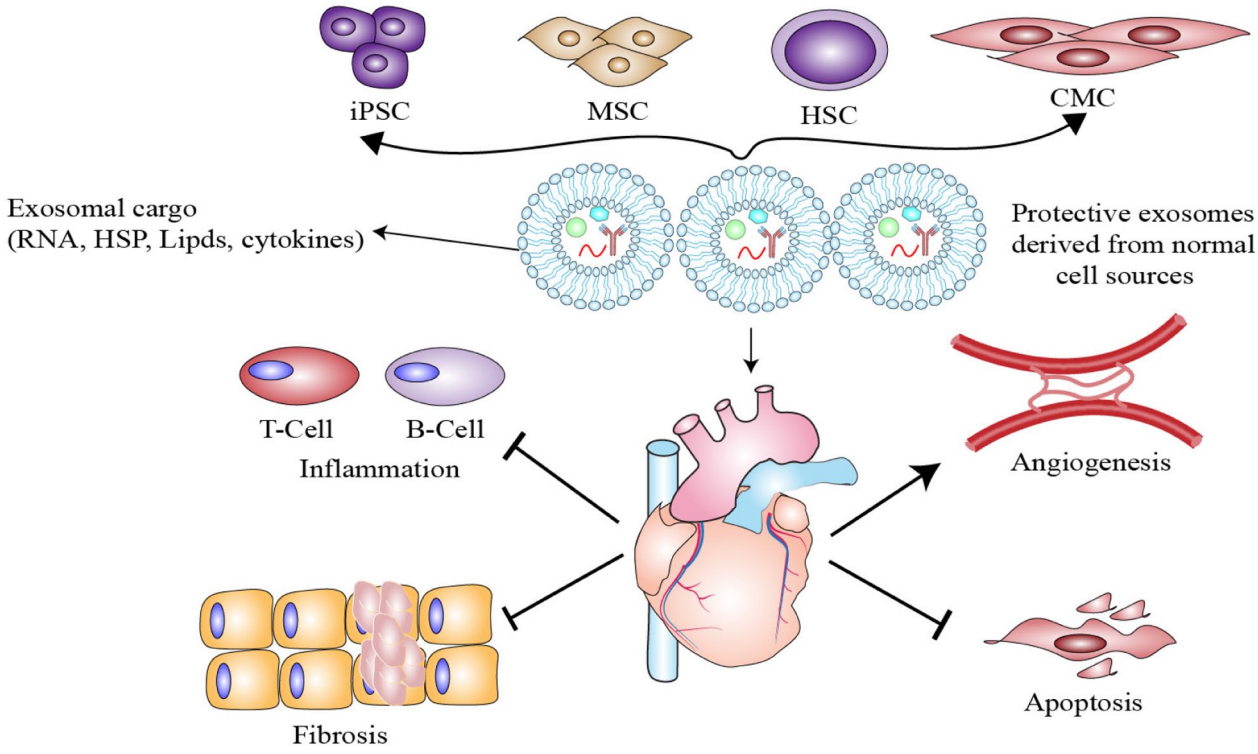

Figure 3. Therapeutic applications of exosomes for cardiac diseases. Exosomes isolated from a variety of normal cells, such as induced pluripotent stem cells (iPSCs), mesenchymal stem cells (MSCs), hematopoietic stem cells (HSCs), and cardiomyocytes (CMCs), have been shown to improve angiogenesis, cardiomyocyte proliferation, and cardiac function and attenuate many cardiac pathological conditions such as fibrosis, apoptosis, inflammation, and oxidative stress.

Therapeutic applications of stem cell–derived exosomes in CVDs

Exosomes are currently being used as therapy for various disease by manipulating on its therapeutic need. We are summarizing how the stem cells were exploited for the treatment of diseases such as myocardial ischemia, apoptosis, hypertrophy, and sepsis (Figure 3).

Myocardial ischemia

Myocardial ischemia is an infirmity where the blood flow to the heart is constricted caused by the blockage of the heart's arteries, preventing the heart muscles from receiving enough oxygen. This condition can ultimately lead to heart attacks and abnormal heart rhythms. Many *in vitro* studies have been performed with stem cell–derived exosomes. Exosomes obtained from cardiosphere-derived cells have been shown to reduce scarring and induce angiogenesis in the pig model of acute myocardial infarction.[95] Hypoxic preconditioning of cardiosphere-derived cells increases the cardioprotective effect of exosomes by promoting angiogenesis through exosomal miRNA-210, miRNA-130a, and miRNA-126. They have also been shown to promote tube formation in human umbilical vein endothelial cells (HUVEC) cells.[96] Exosomes from embryonic stem cells–derived MSCs have been shown to alleviate oxidative stress during myocardial ischemia/reperfusion (MI/R) injury by activating PI3K/Akt pathway. They have been shown to reduce the infarct size, restore bioenergetics, and prevent cardiac remodeling.[97] MSC-derived exosomes have been shown to promote neovascularization, inhibit the inflammation response, and have been shown to refine the cardiac microenvironment by promoting angiogenesis.[98] Exosomes secreted by CXCR4-overexpressing MSCs have been shown to promote cytoprotective effects on cardiomyocytes by mediating the Akt signaling.[99] Exosomes from MSC-derived CPCs have been shown to promote angiogenesis through ERK/Akt signaling, leading to endothelial cell migration and vessel formation. Furthermore, many of pro-angiogenic factors such as extracellular matrix metalloproteinase inducer (EMMPRIN) have been upregulated, indicating the recovery of heart.[100] MSC-derived exosomes have been shown to improve chronic wound healing by regulating Akt, ERK, and STAT3 signaling pathways. They have been shown to promote angiogenesis in HUVEC through enhanced expression of bFGF, vascular endothelial growth factor, and TGF-β.[101] MSC-derived exosomes have been used for MI/R treatment. These exosomes were overexpressing CD47 and protecting them from the mononuclear phagocyte system with the help of miR-21a. Furthermore, these exosomes have been shown to inhibit apoptosis and inflammation, and improve cardiac function.[102] Exosomes obtained from adipose-derived MSC inactivated TGFBR2 and inhibited the phosphorylation of SMAD2 through miR-671.[103] Human umbilical cord-MSC-derived exosomes promoted β-catenin translocation by activating the Wnt pathway, exerting a proangiogenic effect on the rat skin burn model.[104] An exosome spray has been developed that repair postcardiac injury. This has been shown to promote angiomyogenesis, reduce fibrosis, and exhibit efficient cardiac function.[105] Hypoxia-pretreated olfactory mucosa MSC-derived exosomes promoted angiogenesis, tissue

repair, and regeneration via miR-612, displaying a promising strategy for ischemic diseases.[106] MSC-derived exosomes treated with atorvastatin, a popular cardioprotective drug, have been shown to magnify its therapeutic potential in acute myocardial infarction and promote endothelial cell function with long non-coding RNA H19 as its regulator.[107] Co-transplantation of exosomes from adipose-derived stem cells exposed to hypoxia has enhanced grafted tissue neoangiogenesis and survival by regulating vascular endothelial growth factor (VEGF)/vascular endothelial growth factor receptor (VEGF-R) signaling.[108] Thus, stem cell–derived exosomes have potential application in the treatment of myocardial infarction.

Cardiac apoptosis

When cardiomyocytes are exposed to stresses such as myocardial ischemia, hypoxia, and hypertension, they undergo apoptosis and necrosis, resulting in cardiac dysfunction and ultimately heart failure. MSC-derived exosomes overexpressing GATA-4 had higher levels of miR-19a that inhibited cardiomyocyte apoptosis through the activation of the Akt and ERK pathway.[109] Hypoxia-challenged MSC-derived exosomes alleviated apoptosis of cardiac cells in a post myocardial infarction animal model through delivery of miR-210.[110] They also inhibited hypoxia-induced apoptosis in cardiomyocytes through transfer of miR-144 and by inhibiting PTEN and promoting p-AKT expression in cardiac cells.[111] Cardiac stem cell–derived exosomes have been shown to play a pivotal role in ATP generation with the aid of exosomal pyruvate kinase, GAPDH, enolase, and phosphoglucomutase, and thereby inhibiting oxidative stress and cardiac apoptosis.[112] Exosomal miR-210 inhibited cardiomyocyte apoptosis by silencing pro-apoptotic protein tyrosine phosphatase 1B and ephrin A3.[113] Exosomal miR-451 and miR-21 have been shown to obstruct cardiac apoptosis by inhibiting the activity of caspase 3/7.[114] Cardiac myocytes exposed to myocardial injury and treated with MSC-derived exosomes have been shown to promote clathrin-mediated endocytosis of miR-214, which resulted in the inhibition of apoptosis and improvement in the conditions of acute myocardial infarction.[115]

Cardiac hypertrophy

The characteristics of cardiac hypertrophy include the secretion of extracellular matrix and pro-inflammatory cytokines, leading to the proliferation of cardiac fibroblast and myocyte enlargement.[116] MSC-derived exosomes have been shown to attenuate cardiac hypertrophy through inhibiting the proliferation of cardiac fibroblasts and secretion of extracellular matrix and pro-inflammatory cytokines.[116] Intravenous injection of MSC-derived exosomes attenuated inflammation in cardiac microenvironment through modulation of macrophages.[117] Experimentally induced pulmonary hypertension in rats was improved by treatment with MSC-derived exosomes through upregulation of Wnt and BMP proteins and attenuating pulmonary vascular remodeling and lung fibrosis *in vivo*.[118] Bone marrow MSC-derived exosomes protected the heart from cardiac hypertrophy and apoptosis upon pressure overload. Upon induction with angiotensin 2, exosomes also attenuated cardiomyocyte hypertrophy.[119]

Sepsis

Sepsis is a life-threatening medical emergency and refers to the body's extreme response to infection. It causes a broad spectrum of acute myocardial impairment. Exosomal cargoes, such as damage-associated molecular patterns (DAMPs), heat-shock proteins (HSPs), high mobility group box-1 (HMGB1), and a number of extracellular RNAs, have been shown to regulate the sepsis-associated inflammation.[120] It is mainly caused by an unbalanced immune response to infectious agents.[121,122] Upon bacterial infection, the function of the heart starts to reduce, subsequently causing multiple organ failure. Such cascade of events may lead to sepsis-induced cardiomyopathy. MSC-derived exosomal miR-27b attenuated the NF-kB pathway by silencing JMJD3 and by downregulating the expression of pro-inflammatory cytokines and alleviated sepsis *in vitro* and *in vivo*.[123] miR-21 generated by MSC-derived exosomes have been shown to ameliorate sepsis by silencing PDCD4, thereby promoting M2 macrophage polarization.[124] Adipose-derived exosomes have been shown to alleviate sepsis-induced inflammation and multiple organ failure through polarizing macrophages to M2 phenotype. They attenuate ROS accumulation and downregulated the expression of pro-inflammatory cytokines through the regulation of Nrf-2/HO-1 axis.[125] Another important issue associated with sepsis is acute respiratory distress syndrome (ARDS), and macrophages are the pivotal factors of ARDS. BMSC-derived exosomes have been shown to inhibit glycolysis in macrophages, thereby inhibiting the formation of pro-inflammatory M1 phenotype and promoting anti-inflammatory M2 phenotype.[126] BMSC-derived exosomal miR-223 has been shown to exert a cardioprotective effect during polymicrobial sepsis through downregulation of semaphorin-3A and STAT3 leading to decreased inflammation and cell death.[127] In LPS-induced mouse models, platelet-derived exosomes promote inflammation through the release of nitric oxide synthase, NADPH oxidase, and disulfide isomerase, and consequently downregulating an anti-inflammatory miR-223. These platelet-derived exosomes regulate the vascular dysfunction, as observed in sepsis.[128] So, identifying such targets and acting on them might help in the fight against sepsis.

Future perspectives and conclusions

Now, it has become evident that almost all cells secrete exosomes and they have been widely applied in therapeutics. Exosomes have been utilized as biomarkers in many CVDs. Exosome bioengineering has enabled us to enhance the specificity of the target cells and avoid immunogenic effects. Even though several preclinical studies have demonstrated the role of exosomes in CVDs, only two trials have successfully entered into clinical trials. A randomized, single-blind, placebo-controlled, Phase 1, 2 trial evaluates the safety and efficacy of allogenic MSC-derived exosome in improving the disability of patients with acute ischemic stroke (ClinicalTrials.gov Identifier: NCT03384433). In another study, the patients undergoing surgical repair of acute type A aortic dissection (ATAAD) immediately presenting severe multiple organ dysfunction syndrome (MODS) will be treated with exosomes derived from umbilical cord–derived MSCs (NCT04356300).

Several questions regarding exosomes are obscure and are yet to be elucidated. First, we have to evaluate the molecular heterogeneity of exosomes in order to apply them therapeutically on a large scale. We need to identify the functional mechanism involved in cargo sorting, including RNA and protein packaging. Second, we have to map the route taken by the exosomes while delivering paracrine factors. Third, proper isolation and characterization protocol for exosomes need to be established as current methods are not viable. Furthermore, a better understanding on exosome biogenesis and their uptake is needed. When these challenges on the exosomes are being addressed, the full therapeutic potential of exosomes can be unleashed. Taken together, by exploiting the properties of exosomes and stem cells, we can become one step closer to developing non-invasive, cell-free therapeutics for CVDs.

AUTHORS' CONTRIBUTIONS

SM, TU, NG, and JR contributed to conceptualization and literature collection; SM, TU, and SR contributed to original draft preparation; SM, AJP, NG, and JR contributed to writing, reviewing, and editing; and JR contributed to funding acquisition. All authors have read and agreed to the current version of the manuscript.

DECLARATION OF CONFLICTING INTERESTS

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REFERENCES

1. Tsao CW, Aday AW, Almarzooq ZI, Alonso A, Beaton AZ, Bittencourt MS, Boehme AK, Buxton AE, Carson AP, Commodore-Mensah Y. Heart disease and stroke statistics – 2022 update: a report from the American Heart Association. *Circulation* 2022;**145**:e153–639
2. Suzman R, Beard JR, Boerma T, Chatterji S. Health in an ageing world – what do we know? *Lancet* 2015;**385**:484–6
3. Blau HM, Brazelton T, Weimann J. The evolving concept of a stem cell: entity or function? *Cell* 2001;**105**:829–41
4. Yamanaka S. Pluripotent stem cell-based cell therapy – promise and challenges. *Cell Stem Cell* 2020;**27**:523–31
5. Zakrzewski W, Dobrzyński M, Szymonowicz M, Rybak Z. Stem cells: past, present, and future. *Stem Cell Res Ther* 2019;**10**:1–22
6. Lalit PA, Hei DJ, Raval AN, Kamp TJ. Induced pluripotent stem cells for post–myocardial infarction repair: remarkable opportunities and challenges. *Circ Res* 2014;**114**:1328–45
7. Sun S-J, Wei R, Li F, Liao S-Y, Tse H-F. Mesenchymal stromal cell-derived exosomes in cardiac regeneration and repair. *Stem Cell Rep* 2021;**16**:1662–73
8. Menasché P. Skeletal myoblasts and cardiac repair. *J Mol Cell Cardiol* 2008;**45**:545–53
9. Sebastião MJ, Serra M, Pereira R, Palacios I, Gomes-Alves P, Alves PM. Human cardiac progenitor cell activation and regeneration mechanisms: exploring a novel myocardial ischemia/reperfusion in vitro model. *Stem Cell Res Ther* 2019;**10**:77
10. Beliën H, Evens L, Hendrikx M, Bito V, Bronckaers A. Combining stem cells in myocardial infarction: the road to superior repair. *Med Res Rev* 2022;**42**:343–73
11. Yan F, Liu X, Ding H, Zhang W. Paracrine mechanisms of endothelial progenitor cells in vascular repair. *Acta Histochem* 2022;**124**:151833
12. Pittenger MF, Discher DE, Péault BM, Phinney DG, Hare JM, Caplan AI. Mesenchymal stem cell perspective: cell biology to clinical progress. *NPJ Regen Med* 2019;**4**:22–15
13. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. *Science* 1999;**284**:143–7
14. Hare JM, DiFede DL, Rieger AC, Florea V, Landin AM, El-Khorazaty J, Khan A, Mushtaq M, Lowery MH, Byrnes JJ. Randomized comparison of allogeneic versus autologous mesenchymal stem cells for non-ischemic dilated cardiomyopathy: POSEIDON-DCM trial. *J Am Coll Cardiol* 2017;**69**:526–37
15. Hare JM, Fishman JE, Gerstenblith G, Velazquez DLD, Zambrano JP, Suncion VY, Tracy M, Ghersin E, Johnston PV, Brinker JA. Comparison of allogeneic vs autologous bone marrow–derived mesenchymal stem cells delivered by transendocardial injection in patients with ischemic cardiomyopathy: the POSEIDON randomized trial. *JAMA* 2012;**308**:2369–79
16. Butler J, Epstein SE, Greene SJ, Quyyumi AA, Sikora S, Kim RJ, Anderson AS, Wilcox JE, Tankovich NI, Lipinski MJ. Intravenous allogeneic mesenchymal stem cells for nonischemic cardiomyopathy: safety and efficacy results of a phase II-A randomized trial. *Circ Res* 2017;**120**:332–40
17. Karantalis V, Suncion-Loescher VY, Bagno L, Golpanian S, Wolf A, Sanina C, Premer C, Kanelidis AJ, McCall F, Wang B, Balkan W, Rodriguez J, Rosado M, Morales A, Hatzistergos K, Natsumeda M, Margitich I, Schulman IH, Gomes SA, Mushtaq M, DiFede DL, Fishman JE, Pattany P, Zambrano JP, Heldman AW, Hare JM. Synergistic effects of combined cell therapy for chronic ischemic cardiomyopathy. *J Am Coll Cardiol* 2015;**66**:1990–9
18. Karantalis V, DiFede DL, Gerstenblith G, Pham S, Symes J, Zambrano JP, Fishman J, Pattany P, McNiece I, Conte J, Schulman S, Wu K, Shah A, Breton E, Davis-Sproul J, Schwarz R, Feigenbaum G, Mushtaq M, Suncion VY, Lardo AC, Borrello I, Mendizabal A, Karas TZ, Byrnes J, Lowery M, Heldman AW, Hare JM. Autologous mesenchymal stem cells produce concordant improvements in regional function, tissue perfusion, and fibrotic burden when administered to patients undergoing coronary artery bypass grafting: the prospective randomized study of mesenchymal stem cell therapy in patients undergoing cardiac surgery (PROMETHEUS) trial. *Circ Res* 2014;**114**:1302–10
19. Eschenhagen T, Bolli R, Braun T, Field LJ, Fleischmann BK, Frisén J, Giacca M, Hare JM, Houser S, Lee RT, Marbán E, Martin JF, Molkentin JD, Murry CE, Riley PR, Ruiz-Lozano P, Sadek HA, Sussman MA, Hill JA. Cardiomyocyte regeneration: a consensus statement. *Circulation* 2017;**136**:680–6
20. Mathiasen AB, Qayyum AA, Jørgensen E, Helqvist S, Fischer-Nielsen A, Kofoed KF, Haack-Sørensen M, Ekblond A, Kastrup J. Bone marrow-derived mesenchymal stromal cell treatment in patients with severe ischaemic heart failure: a randomized placebo-controlled trial (MSC-HF trial). *Eur Heart J* 2015;**36**:1744–53
21. Perin EC, Borow KM, Silva GV, DeMaria AN, Marroquin OC, Huang PP, Traverse JH, Krum H, Skerrett D, Zheng Y, Willerson JT, Itescu S, Henry TD. A phase II dose-escalation study of allogeneic mesenchymal precursor cells in patients with ischemic or nonischemic heart failure. *Circ Res* 2015;**117**:576–84
22. Arienti G, Carlini E, Verdacchi R, Cosmi EV, Palmerini CA. Prostasome to sperm transfer of CD13/aminopeptidase N (EC 3.4.11.2). *Biochim Biophys Acta* 1997;**1336**:533–8

23. Frenette G, Sullivan R. Prostasome-like particles are involved in the transfer of P25b from the bovine epididymal fluid to the sperm surface. *Mol Reprod Dev* 2001;**59**:115–21
24. Harding C, Heuser J, Stahl P. Endocytosis and intracellular processing of transferrin and colloidal gold-transferrin in rat reticulocytes: demonstration of a pathway for receptor shedding. *Eur J Cell Biol* 1984;**35**:256–63
25. Pan BT, Johnstone RM. Fate of the transferrin receptor during maturation of sheep reticulocytes in vitro: selective externalization of the receptor. *Cell* 1983;**33**:967–78
26. Bakhshian Nik A, Hutcheson JD, Aikawa E. Extracellular vesicles as mediators of cardiovascular calcification. *Front Cardiovasc Med* 2017;**4**:78
27. Anderson HC, Garimella R, Tague SE. The role of matrix vesicles in growth plate development and biomineralization. *Front Biosci* 2005; **10**:822–37
28. Street JM, Barran PE, Mackay CL, Weidt S, Balmforth C, Walsh TS, Chalmers RT, Webb DJ, Dear JW. Identification and proteomic profiling of exosomes in human cerebrospinal fluid. *J Transl Med* 2012;**10**:5
29. Chen C, Skog J, Hsu CH, Lessard RT, Balaj L, Wurdinger T, Carter BS, Breakefield XO, Toner M, Irimia D. Microfluidic isolation and transcriptome analysis of serum microvesicles. *Lab Chip* 2010;**10**:505–11
30. Ma ZJ, Yang JJ, Lu YB, Liu ZY, Wang XX. Mesenchymal stem cell-derived exosomes: toward cell-free therapeutic strategies in regenerative medicine. *World J Stem Cells* 2020;**12**:814–40
31. Zhang J, Rong Y, Luo C, Cui W. Bone marrow mesenchymal stem cell-derived exosomes prevent osteoarthritis by regulating synovial macrophage polarization. *Aging* 2020;**12**:25138–52
32. Yang X, Yang J, Lei P, Wen T. LncRNA MALAT1 shuttled by bone marrow-derived mesenchymal stem cells-secreted exosomes alleviates osteoporosis through mediating microRNA-34c/SATB2 axis. *Aging* 2019;**11**:8777–91
33. Sengupta V, Sengupta S, Lazo A, Woods P, Nolan A, Bremer N. Exosomes derived from bone marrow mesenchymal stem cells as treatment for severe COVID-19. *Stem Cells Dev* 2020;**29**:747–54
34. Ma T, Fu B, Yang X, Xiao Y, Pan M. Adipose mesenchymal stem cell-derived exosomes promote cell proliferation, migration, and inhibit cell apoptosis via Wnt/β-catenin signaling in cutaneous wound healing. *J Cell Biochem* 2019;**120**:10847–54
35. Xiao C, Wang K, Xu Y, Hu H, Zhang N, Wang Y, Zhong Z, Zhao J, Li Q, Zhu D, Ke C, Zhong S, Wu X, Yu H, Zhu W, Chen J, Zhang J, Wang J, Hu X. Transplanted mesenchymal stem cells reduce autophagic flux in infarcted hearts via the exosomal transfer of miR-125b. *Circ Res* 2018;**123**:564–78
36. Peng Y, Zhao JL, Peng ZY, Xu WF, Yu GL. Exosomal miR-25-3p from mesenchymal stem cells alleviates myocardial infarction by targeting pro-apoptotic proteins and EZH2. *Cell Death Dis* 2020;**11**:317
37. Ratajczak J, Kucia M, Mierzejewska K, Marlicz W, Pietrzkowski Z, Wojakowski W, Greco NJ, Tendera M, Ratajczak MZ. Paracrine pro-angiopoietic effects of human umbilical cord blood-derived purified CD133+ cells – implications for stem cell therapies in regenerative medicine. *Stem Cells Dev* 2013;**22**:422–30
38. Seeger FH, Zeiher AM, Dimmeler S. MicroRNAs in stem cell function and regenerative therapy of the heart. *Arterioscler Thromb Vasc Biol* 2013;**33**:1739–46
39. Liu H, Gao W, Yuan J, Wu C, Yao K, Zhang L, Ma L, Zhu J, Zou Y, Ge J. Exosomes derived from dendritic cells improve cardiac function via activation of CD4(+) T lymphocytes after myocardial infarction. *J Mol Cell Cardiol* 2016;**91**:123–33
40. Krenning G, van Luyn MJ, Harmsen MC. Endothelial progenitor cell-based neovascularization: implications for therapy. *Trends Mol Med* 2009;**15**:180–9
41. Li M, Soder R, Abhyankar S, Abdelhakim H, Braun MW, Trinidad CV, Pathak HB, Pessetto Z, Deighan C, Ganguly S, Dawn B, McGuirk J, Dunavin N, Godwin AK. WJMSC-derived small extracellular vesicle enhance T cell suppression through PD-L1. *J Extracell Vesicles* 2021; **10**:e12067
42. Jiang S, Tian G, Yang Z, Gao X, Wang F, Li J, Tian Z, Huang B, Wei F, Sang X, Shao L, Zhou J, Wang Z, Liu S, Sui X, Guo Q, Guo W, Li X. Enhancement of acellular cartilage matrix scaffold by Wharton's jelly mesenchymal stem cell-derived exosomes to promote osteochondral regeneration. *Bioact Mater* 2021;**6**:2711–28
43. Chopra N, Dutt Arya B, Jain N, Yadav P, Wajid S, Singh SP, Choudhury S. Biophysical characterization and drug delivery potential of exosomes from human Wharton's jelly-derived mesenchymal stem cells. *ACS Omega* 2019;**4**:13143–52
44. Khan M, Nickoloff E, Abramova T, Johnson J, Verma SK, Krishnamurthy P, Mackie AR, Vaughan E, Garikipati VNS, Benedict C, Ramirez V, Lambers E, Ito A, Gao E, Misener S, Luongo T, Elrod J, Qin G, Houser SR, Koch WJ, Kishore R. Embryonic stem cell-derived exosomes promote endogenous repair mechanisms and enhance cardiac function following myocardial infarction. *Circ Res* 2015;**117**:52–64
45. Sabapathy V, Kumar S. hi PSC-derived iMSCs: NextGen MSCs as an advanced therapeutically active cell resource for regenerative medicine. *J Cell Mol Med* 2016;**20**:1571–88
46. Skotland T, Sandvig K, Llorente A. Lipids in exosomes: current knowledge and the way forward. *Prog Lipid Res* 2017;**66**:30–41
47. Shimoda A, Tahara Y, Sawada SI, Sasaki Y, Akiyoshi K. Glycan profiling analysis using evanescent-field fluorescence-assisted lectin array: importance of sugar recognition for cellular uptake of exosomes from mesenchymal stem cells. *Biochem Biophys Res Commun* 2017;**491**: 701–7
48. Pegtel DM, Gould SJ. Exosomes. *Annu Rev Biochem* 2019;**88**:487–514
49. Mathivanan S, Fahner CJ, Reid GE, Simpson RJ. ExoCarta 2012: database of exosomal proteins, RNA and lipids. *Nucleic Acids Res* 2012; **40**:D1241–124
50. Hemler ME. Tetraspanin proteins mediate cellular penetration, invasion, and fusion events and define a novel type of membrane microdomain. *Annu Rev Cell Dev Biol* 2003;**19**:397–422
51. Liang Y, Eng WS, Colquhoun DR, Dinglasan RR, Graham DR, Mahal LK. Complex N-linked glycans serve as a determinant for exosome/microvesicle cargo recruitment. *J Biol Chem* 2014;**289**:32526–37
52. Reddy VS, Madala SK, Trinath J, Reddy GB. Extracellular small heat shock proteins: exosomal biogenesis and function. *Cell Stress Chaperones* 2018;**23**:441–54
53. Radulovic M, Stenmark H. ESCRTs in membrane sealing. *Biochem Soc Trans* 2018;**46**:773–8
54. Ashley J, Cordy B, Lucia D, Fradkin LG, Budnik V, Thomson T. Retrovirus-like gag protein arc1 binds RNA and traffics across synaptic boutons. *Cell* 2018;**172**:262–74
55. Baietti MF, Zhang Z, Mortier E, Melchior A, Degeest G, Geeraerts A, Ivarsson Y, Depoortere F, Coomans C, Vermeiren E, Zimmermann P, David G. Syndecan-syntenin-ALIX regulates the biogenesis of exosomes. *Nat Cell Biol* 2012;**14**:677–85
56. An Q, van Bel AJ, Hückelhoven R. Do plant cells secrete exosomes derived from multivesicular bodies. *Plant Signal Behav* 2007;**2**:4–7
57. Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ, Lötvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat Cell Biol* 2007;**9**: 654–9
58. Jaiswal R, Gong J, Sambasivam S, Combes V, Mathys JM, Davey R, Grau GE, Bebawy M. Microparticle-associated nucleic acids mediate trait dominance in cancer. *FASEB J* 2012;**26**:420–9
59. Thakur BK, Zhang H, Becker A, Matei I, Huang Y, Costa-Silva B, Zheng Y, Hoshino A, Brazier H, Xiang J, Williams C, Rodriguez-Barrueco R, Silva JM, Zhang W, Hearn S, Elemento O, Paknejad N, Manova-Todorova K, Welte K, Bromberg J, Peinado H, Lyden D. Double-stranded DNA in exosomes: a novel biomarker in cancer detection. *Cell Res* 2014;**24**:766–9
60. Wollert T, Hurley JH. Molecular mechanism of multivesicular body biogenesis by ESCRT complexes. *Nature* 2010;**464**:864–9
61. Samanta S, Rajasingh S, Drosos N, Zhou Z, Dawn B, Rajasingh J. Exosomes: new molecular targets of diseases. *Acta Pharmacol Sin* 2018; **39**:501–13
62. Möbius W, Ohno-Iwashita Y, van Donselaar EG, Oorschot VM, Shimada Y, Fujimoto T, Heijnen HF, Geuze HJ, Slot JW. Immunoelectron microscopic localization of cholesterol using biotinylated and non-cytolytic perfringolysin O. *J Histochem Cytochem* 2002;**50**:43–55

63. Shao H, Chung J, Balaj L, Charest A, Bigner DD, Carter BS, Hochberg FH, Breakefield XO, Weissleder R, Lee H. Protein typing of circulating microvesicles allows real-time monitoring of glioblastoma therapy. *Nat Med* 2012;**18**:1835–40
64. Wang Q, Yu J, Kadungure T, Beyene J, Zhang H, Lu Q. ARMMs as a versatile platform for intracellular delivery of macromolecules. *Nat Commun* 2018;**9**:960
65. Nkwe DO, Pelchen-Matthews A, Burden JJ, Collinson LM, Marsh M. The intracellular plasma membrane-connected compartment in the assembly of HIV-1 in human macrophages. *BMC Biol* 2016;**14**:50
66. Murphy DE, de Jong OG, Brouwer M, Wood MJ, Lavieu G, Schiffelers RM, Vader P. Extracellular vesicle-based therapeutics: natural versus engineered targeting and trafficking. *Exp Mol Med* 2019;**51**:1–12
67. van Dongen HM, Masoumi N, Witwer KW, Pegtel DM. Extracellular vesicles exploit viral entry routes for cargo delivery. *Microbiol Mol Biol Rev* 2016;**80**:369–86
68. Sahoo S, Kariya T, Ishikawa K. Targeted delivery of therapeutic agents to the heart. *Nat Rev Cardiol* 2021;**18**:389–99
69. Herrmann IK, Wood MJA, Fuhrmann G. Extracellular vesicles as a next- generation drug delivery platform. *Nat Nanotechnol* 2021;**16**: 748–59
70. Khan H, Pan JJ, Li Y, Zhang Z, Yang GY. Native and bioengineered exosomes for ischemic stroke therapy. *Front Cell Dev Biol* 2021;**9**: 619565
71. Suk JS, Xu Q, Kim N, Hanes J, Ensign LM. PEGylation as a strategy for improving nanoparticle-based drug and gene delivery. *Adv Drug Deliv Rev* 2016;**99**:28–51
72. de Abreu RC, Fernandes H, da Costa Martins PA, Sahoo S, Emanueli C, Ferreira L. Native and bioengineered extracellular vesicles for cardiovascular therapeutics. *Nat Rev Cardiol* 2020;**17**:685–97
73. Shin M, Lee HA, Lee M, Shin Y, Song JJ, Kang SW, Nam DH, Jeon EJ, Cho M, Do M, Park S, Lee MS, Jang JH, Cho SW, Kim KS, Lee H. Targeting protein and peptide therapeutics to the heart via tannic acid modification. *Nat Biomed Eng* 2018;**2**:304–17
74. Antes TJ, Middleton RC, Luther KM, Ijichi T, Peck KA, Liu WJ, Valle J, Echavez AK, Marbán E. Targeting extracellular vesicles to injured tissue using membrane cloaking and surface display. *J Nanobiotechnology* 2018;**16**:61
75. Vandergriff A, Huang K, Shen D, Hu S, Hensley MT, Caranasos TG, Qian L, Cheng K. Targeting regenerative exosomes to myocardial infarction using cardiac homing peptide. *Theranostics* 2018;**8**:1869–78
76. Tian T, Zhang HX, He CP, Fan S, Zhu YL, Qi C, Huang NP, Xiao ZD, Lu ZH, Tannous BA, Gao J. Surface functionalized exosomes as targeted drug delivery vehicles for cerebral ischemia therapy. *Biomaterials* 2018;**150**:137–49
77. Hwang DW, Jo MJ, Lee JH, Kang H, Bao K, Hu S, Baek Y, Moon HG, Lee DS, Kashiwagi S, Henary M, Choi HS. Chemical modulation of bioengineered exosomes for tissue-specific biodistribution. *Adv Ther* 2019;**2**:1900111.
78. Hein CD, Liu XM, Wang D. Click chemistry, a powerful tool for pharmaceutical sciences. *Pharm Res* 2008;**25**:2216–30
79. Wang X, Chen Y, Zhao Z, Meng Q, Yu Y, Sun J, Yang Z, Chen Y, Li J, Ma T, Liu H, Li Z, Yang J, Shen Z. Engineered exosomes with ischemic myocardium-targeting peptide for targeted therapy in myocardial infarction. *J Am Heart Assoc* 2018;**7**:e008737
80. Nakase I, Futaki S. Combined treatment with a pH-sensitive fusogenic peptide and cationic lipids achieves enhanced cytosolic delivery of exosomes. *Sci Rep* 2015;**5**:10112
81. Nakase I, Noguchi K, Aoki A, Takatani-Nakase T, Fujii I, Futaki S. Arginine-rich cell-penetrating peptide-modified extracellular vesicles for active macropinocytosis induction and efficient intracellular delivery. *Sci Rep* 2017;**7**:1991
82. Tuerk C, Gold L. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. *Science* 1990;**249**:505–10
83. Harishkumar M, Radha M, Yuichi N, Muthukalianan GK, Kaoru O, Shiomori K, Sakai K, Nozomi W. Designer exosomes: smart nanocommunication tools for translational medicine. *Bioengineering* 2021; **8**:158
84. Ribeiro-Rodrigues TM, Laundos TL, Pereira-Carvalho R, Batista-Almeida D, Pereira R, Coelho-Santos V, Silva AP, Fernandes R, Zuzarte M, Enguita FJ, Costa MC, Pinto-do-Ó P, Pinto MT, Gouveia P, Ferreira L, Mason JC, Pereira P, Kwak BR, Nascimento DS, Girão H. Exosomes secreted by cardiomyocytes subjected to ischaemia promote cardiac angiogenesis. *Cardiovasc Res* 2017;**113**:1338–50
85. Zhang Y, Liu D, Chen X, Li J, Li L, Bian Z, Sun F, Lu J, Yin Y, Cai X, Sun Q, Wang K, Ba Y, Wang Q, Wang D, Yang J, Liu P, Xu T, Yan Q, Zhang J, Zen K, Zhang CY. Secreted monocytic miR-150 enhances targeted endothelial cell migration. *Mol Cell* 2010;**39**:133–44
86. Gray WD, French KM, Ghosh-Choudhary S, Maxwell JT, Brown ME, Platt MO, Searles CD, Davis ME. Identification of therapeutic covariant microRNA clusters in hypoxia-treated cardiac progenitor cell exosomes using systems biology. *Circ Res* 2015;**116**:255–63
87. He B, Xiao J, Ren AJ, Zhang YF, Zhang H, Chen M, Xie B, Gao XG, Wang YW. Role of miR-1 and miR-133a in myocardial ischemic postconditioning. *J Biomed Sci* 2011;**18**:22
88. Castoldi G, Di Gioia CR, Bombardi C, Catalucci D, Corradi B, Gualazzi MG, Leopizzi M, Mancini M, Zerbini G, Condorelli G, Stella A. MiR-133a regulates collagen 1A1: potential role of miR-133a in myocardial fibrosis in angiotensin II-dependent hypertension. *J Cell Physiol* 2012;**227**:850–6
89. Izarra A, Moscoso I, Levent E, Cañón S, Cerrada I, Díez-Juan A, Blanca V, Núñez-Gil IJ, Valiente I, Ruíz-Sauri A, Sepúlveda P, Tiburcy M, Zimmermann WH, Bernad A. miR-133a enhances the protective capacity of cardiac progenitors cells after myocardial infarction. *Stem Cell Rep* 2014;**3**:1029–42
90. Li S, Xiao FY, Shan PR, Su L, Chen DL, Ding JY, Wang ZQ. Overexpression of microRNA-133a inhibits ischemia-reperfusion-induced cardiomyocyte apoptosis by targeting DAPK2. *J Hum Genet* 2015;**60**: 709–16
91. Alvarez-Erviti L, Seow Y, Yin H, Betts C, Lakhal S, Wood MJ. Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. *Nat Biotechnol* 2011;**29**:341–5
92. György B, Fitzpatrick Z, Crommentuijn MH, Mu D, Maguire CA. Naturally enveloped AAV vectors for shielding neutralizing antibodies and robust gene delivery in vivo. *Biomaterials* 2014;**35**:7598–609
93. Liu B, Li Z, Huang S, Yan B, He S, Chen F, Liang Y. AAV-containing exosomes as a novel vector for improved gene delivery to lung cancer cells. *Front Cell Dev Biol* 2021;**9**:707607
94. Nolte-'t Hoen E, Cremer T, Gallo RC, Margolis LB. Extracellular vesicles and viruses: are they close relatives? *Proc Natl Acad Sci U S A* 2016;**113**:9155–61
95. Gallet R, Dawkins J, Valle J, Simsolo E, de Couto G, Middleton R, Tseliou E, Luthringer D, Kreke M, Smith RR, Marbán L, Ghaleb B, Marbán E. Exosomes secreted by cardiosphere-derived cells reduce scarring, attenuate adverse remodelling, and improve function in acute and chronic porcine myocardial infarction. *Eur Heart J* 2017;**38**: 201–11
96. Namazi H, Mohit E, Namazi I, Rajabi S, Samadian A, Hajizadeh-Saffar E, Aghdami N, Baharvand H. Exosomes secreted by hypoxic cardiosphere-derived cells enhance tube formation and increase pro-angiogenic miRNA. *J Cell Biochem* 2018;**119**:4150–60
97. Arslan F, Lai RC, Smeets MB, Akeroyd L, Choo A, Aguor EN, Timmers L, van Rijen HV, Doevendans PA, Pasterkamp G, Lim SK, de Kleijn DP. Mesenchymal stem cell-derived exosomes increase ATP levels, decrease oxidative stress and activate PI3K/Akt pathway to enhance myocardial viability and prevent adverse remodeling after myocardial ischemia/reperfusion injury. *Stem Cell Res* 2013;**10**: 301–12
98. Teng X, Chen L, Chen W, Yang J, Yang Z, Shen Z. Mesenchymal stem cell-derived exosomes improve the microenvironment of infarcted myocardium contributing to angiogenesis and anti-inflammation. *Cell Physiol Biochem* 2015;**37**:2415–24
99. Kang K, Ma R, Cai W, Huang W, Paul C, Liang J, Wang Y, Zhao T, Kim HW, Xu M, Millard RW, Wen Z, Wang Y. Exosomes secreted from CXCR4 overexpressing mesenchymal stem cells promote cardioprotection via Akt signaling pathway following myocardial infarction. *Stem Cells Int* 2015;**2015**:659890

100. Vrijsen KR, Maring JA, Chamuleau SA, Verhage V, Mol EA, Deddens JC, Metz CH, Lodder K, van Eeuwijk EC, van Dommelen SM, Doevendans PA, Smits AM, Goumans MJ, Sluijter JP. Exosomes from cardiomyocyte progenitor cells and mesenchymal stem cells stimulate angiogenesis via EMMPRIN. *Adv Healthc Mater* 2016;**5**:2555–65
101. Shabbir A, Cox A, Rodriguez-Menocal L, Salgado M, Van Badiavas E. Mesenchymal stem cell exosomes induce proliferation and migration of normal and chronic wound fibroblasts, and enhance angiogenesis in vitro. *Stem Cells Dev* 2015;**24**:1635–47
102. Wei Z, Chen Z, Zhao Y, Fan F, Xiong W, Song S, Yin Y, Hu J, Yang K, Yang L, Xu B, Ge J. Mononuclear phagocyte system blockade using extracellular vesicles modified with CD47 on membrane surface for myocardial infarction reperfusion injury treatment. *Biomaterials* 2021;**275**:121000
103. Wang X, Zhu Y, Wu C, Liu W, He Y, Yang Q. Adipose-derived mesenchymal stem cells-derived exosomes carry microRNA-671 to alleviate myocardial infarction through Inactivating the TGFBR2/Smad2 Axis. *Inflammation* 2021;**44**:1815–30
104. Zhang B, Wu X, Zhang X, Sun Y, Yan Y, Shi H, Zhu Y, Wu L, Pan Z, Zhu W, Qian H, Xu W. Human umbilical cord mesenchymal stem cell exosomes enhance angiogenesis through the Wnt4/β-catenin pathway. *Stem Cells Transl Med* 2015;**4**:513–22
105. Yao J, Huang K, Zhu D, Chen T, Jiang Y, Zhang J, Mi L, Xuan H, Hu S, Li J, Zhou Y, Cheng K. A minimally invasive exosome spray repairs heart after myocardial infarction. *ACS Nano* 2021;**15**:11099–111
106. Ge L, Xun C, Li W, Jin S, Liu Z, Zhuo Y, Duan D, Hu Z, Chen P, Lu M. Extracellular vesicles derived from hypoxia-preconditioned olfactory mucosa mesenchymal stem cells enhance angiogenesis via miR-612. *J Nanobiotechnology* 2021;**19**:380
107. Huang P, Wang L, Li Q, Tian X, Xu J, Xu J, Xiong Y, Chen G, Qian H, Jin C, Yu Y, Cheng K, Qian L, Yang Y. Atorvastatin enhances the therapeutic efficacy of mesenchymal stem cells-derived exosomes in acute myocardial infarction via up-regulating long non-coding RNA H19. *Cardiovasc Res* 2020;**116**:353–67
108. Han Y, Ren J, Bai Y, Pei X, Han Y. Exosomes from hypoxia-treated human adipose-derived mesenchymal stem cells enhance angiogenesis through VEGF/VEGF-R. *Int J Biochem Cell Biol* 2019;**109**:59–68
109. Yu B, Kim HW, Gong M, Wang J, Millard RW, Wang Y, Ashraf M, Xu M. Exosomes secreted from GATA-4 overexpressing mesenchymal stem cells serve as a reservoir of anti-apoptotic microRNAs for cardioprotection. *Int J Cardiol* 2015;**182**:349–60
110. Cheng H, Chang S, Xu R, Chen L, Song X, Wu J, Qian J, Zou Y, Ma J. Hypoxia-challenged MSC-derived exosomes deliver miR-210 to attenuate post-infarction cardiac apoptosis. *Stem Cell Res Ther* 2020;**11**:224
111. Wen Z, Mai Z, Zhu X, Wu T, Chen Y, Geng D, Wang J. Mesenchymal stem cell-derived exosomes ameliorate cardiomyocyte apoptosis in hypoxic conditions through microRNA144 by targeting the PTEN/AKT pathway. *Stem Cell Res Ther* 2020;**11**:36
112. Chen L, Wang Y, Pan Y, Zhang L, Shen C, Qin G, Ashraf M, Weintraub N, Ma G, Tang Y. Cardiac progenitor-derived exosomes protect ischemic myocardium from acute ischemia/reperfusion injury. *Biochem Biophys Res Commun* 2013;**431**:566–71
113. Barile L, Lionetti V, Cervio E, Matteucci M, Gherghiceanu M, Popescu LM, Torre T, Siclari F, Moccetti T, Vassalli G. Extracellular vesicles from human cardiac progenitor cells inhibit cardiomyocyte apoptosis and improve cardiac function after myocardial infarction. *Cardiovasc Res* 2014;**103**:530–41
114. Xiao J, Pan Y, Li X, Yang X, Feng Y, Tan H, Jiang L, Feng J, Yu X. Cardiac progenitor cell-derived exosomes prevent cardiomyocytes apoptosis through exosomal miR-21 by targeting PDCD4. *Cell Death Dis* 2016;**7**:e2277–1277
115. Eguchi S, Takefuji M, Sakaguchi T, Ishihama S, Mori Y, Tsuda T, Takikawa T, Yoshida T, Ohashi K, Shimizu Y, Hayashida R, Kondo K, Bando YK, Ouchi N, Murohara T. Cardiomyocytes capture stem cell-derived, anti-apoptotic microRNA-214 via clathrin-mediated endocytosis in acute myocardial infarction. *J Biol Chem* 2019;**294**:11665–74
116. Ibrahim A, Marbán E. Exosomes: fundamental biology and roles in cardiovascular physiology. *Annu Rev Physiol* 2016;**78**:67–83
117. Sun X, Shan A, Wei Z, Xu B. Intravenous mesenchymal stem cell-derived exosomes ameliorate myocardial inflammation in the dilated cardiomyopathy. *Biochem Biophys Res Commun* 2018;**503**:2611–8
118. Zhang Z, Ge L, Zhang S, Wang J, Jiang W, Xin Q, Luan Y. The protective effects of MSC-EXO against pulmonary hypertension through regulating Wnt5a/BMP signalling pathway. *J Cell Mol Med* 2020;**24**:13938–48
119. Chen F, Li X, Zhao J, Geng J, Xie J, Xu B. Bone marrow mesenchymal stem cell-derived exosomes attenuate cardiac hypertrophy and fibrosis in pressure overload induced remodeling. *In Vitro Cell Dev Biol Anim* 2020;**56**:567–76
120. Murao A, Brenner M, Aziz M, Wang P. Exosomes in sepsis. *Front Immunol* 2020;**11**:2140
121. Hotchkiss RS, Karl IE. The pathophysiology and treatment of sepsis. *N Engl J Med* 2003;**348**:138–50
122. Habimana R, Choi I, Cho HJ, Kim D, Lee K, Jeong I. Sepsis-induced cardiac dysfunction: a review of pathophysiology. *Acute Crit Care* 2020;**35**:57–66
123. Sun J, Sun X, Chen J, Liao X, He Y, Wang J, Chen R, Hu S, Qiu C. microRNA-27b shuttled by mesenchymal stem cell-derived exosomes prevents sepsis by targeting JMJD3 and downregulating NF-κB signaling pathway. *Stem Cell Res Ther* 2021;**12**:14
124. Yao M, Cui B, Zhang W, Ma W, Zhao G, Xing L. Exosomal miR-21 secreted by IL-1β-primed-mesenchymal stem cells induces macrophage M2 polarization and ameliorates sepsis. *Life Sci* 2021;**264**:118658
125. Shen K, Jia Y, Wang X, Zhang J, Liu K, Wang J, Cai W, Li J, Li S, Zhao M, Wang Y, Hu D. Exosomes from adipose-derived stem cells alleviate the inflammation and oxidative stress via regulating Nrf2/HO-1 axis in macrophages. *Free Radic Biol Med* 2021;**165**:54–66
126. Deng H, Wu L, Liu M, Zhu L, Chen Y, Zhou H, Shi X, Wei J, Zheng L, Hu X, Wang M, He Z, Lv X, Yang H. Bone marrow mesenchymal stem cell-derived exosomes attenuate LPS-induced ARDS by modulating macrophage polarization through inhibiting glycolysis in macrophages. *Shock* 2020;**54**:828–43
127. Wang X, Gu H, Qin D, Yang L, Huang W, Essandoh K, Wang Y, Caldwell CC, Peng T, Zingarelli B, Fan GC. Exosomal miR-223 contributes to mesenchymal stem cell-elicited cardioprotection in polymicrobial sepsis. *Sci Rep* 2015;**5**:13721
128. Gambim MH, do Carmo Ade O, Marti L, Veríssimo-Filho S, Lopes LR, Janiszewski M. Platelet-derived exosomes induce endothelial cell apoptosis through peroxynitrite generation: experimental evidence for a novel mechanism of septic vascular dysfunction. *Crit Care* 2007;**11**:R107