The Use of Secretome and Exosomes in Cardiovascular Diseases

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ABSTRACT

Cardiovascular diseases (CVDs) remain a leading cause of morbidity and mortality worldwide. Recent advancements in regenerative medicine have unveiled the promising roles of secretome and exosomes in the treatment of CVDs. In this article, we aim to understand the roles of secretome and exosomes in the cardiovascular system, both in physiological and pathological conditions, and explore the broad applications of secretome and exosomes in mitigating CVD progression. Secretome and exosomes, which play crucial roles in intercellular communication, tissue repair, and immunomodulation, have shown potential in reducing cardiovascular disease progression by inhibiting inflammation, promoting blood vessel growth, and regulating biological mechanisms. Further research is needed to maximize their use in advanced cardiovascular therapy.

Keywords: *exosome, secretome, cardiovascular disease.*

INTRODUCTION

Cardiovascular diseases (CVDs) remain a leading cause of morbidity and mortality on a global scale. In 2017, CVDs were responsible for a cumulative total of 17.8 million recorded deaths; by 2030, CVDs are expected to cause more than 22.2 million deaths.[1] Current approaches for the treatment of CVDs include pharmacological treatments, cardiovascular interventions, and immunopathophysiological strategies. Unfortunately, current therapeutic strategies have not been able to solve the problem of CVDs. Therefore, various therapeutic methods have been developed to answer these challenges. One strategy involves using the paracrine actions of stem cells to facilitate cardiac regeneration, known as *cell-free therapy*. Cell-free therapy is a treatment method that utilizes the paracrine effects of stem cell products, including secretome and exosomes.[2]

The MSC secretome includes an extensive range of trophic and immunomodulatory cytokines that are released by mesenchymal stem cells (MSCs).[3] The secretome refers to the proteins, microRNAs, and vesicles secreted by cells into the extracellular space, including exosomes, microvesicles, chemokines, growth factors, proteases, autocrine and paracrine hormones, coagulation factors, and their constituent products. The secretome plays an important role in cell signaling, cell communication, and cell migration.[4]

Exosomes are a subtype of extracellular vesicles (EVs) released by cells, along with microvesicles and apoptotic bodies. The subtypes can be distinguished based on their dimensions, composition, and method of development inside cells. Exosomes have a diameter ranging from 25

to 200 nm and are produced within multivesicular structures located within the endocytic pathway. Microvesicles have a diameter ranging from 100 to 1000 nm and are created when the plasma membrane bulges outward and then pinches off. On the other hand, apoptotic bodies are much larger, with a size greater than 1000 nm, and are formed when the membrane of apoptotic cells forms blebs. However, EV research has been impeded by a lack of consistent nomenclature and criteria to classify, isolate, and identify EV subtypes.[5]

Exosomes are considered appropriate therapeutic instruments for cardiac regenerative medicine because of their functional characteristics in comparison to microvesicles and apoptotic bodies. Exosomes are continuously formed from late endosomes through inward budding of the multivesicular body (MVB) membrane, creating intraluminal vesicles (ILVs) within large MVBs. During this process, some proteins and cytosolic components are taken up by and contained inside ILVs. Upon fusion of ILVs with the plasma membrane, the majority are released into the extracellular space and are referred to as exosomes. The content of exosomes varies depending on the cell and includes proteins, lipids, and noncoding RNA such as messenger RNA (mRNA) and microRNA (miRNA). These contents, which represent the pathophysiological state of the secreting cell, are essential for intercellular communication.[6,7]

Biological Roles of Secretome and Exosomes in Cardiovascular Diseases

The secretome is essential for the optimal functioning of the cardiovascular system through the action of secreted molecules. The secretome plays a significant role in facilitating communication between cells (**Figure 1**). Secretome includes proteins, microRNAs, and vesicles that are released by the cell. Cardiomyocytes express secretome in response to stress, serving as a mode of adaptation or communication. The released secretome has a role in modulating renin-angiotensin system

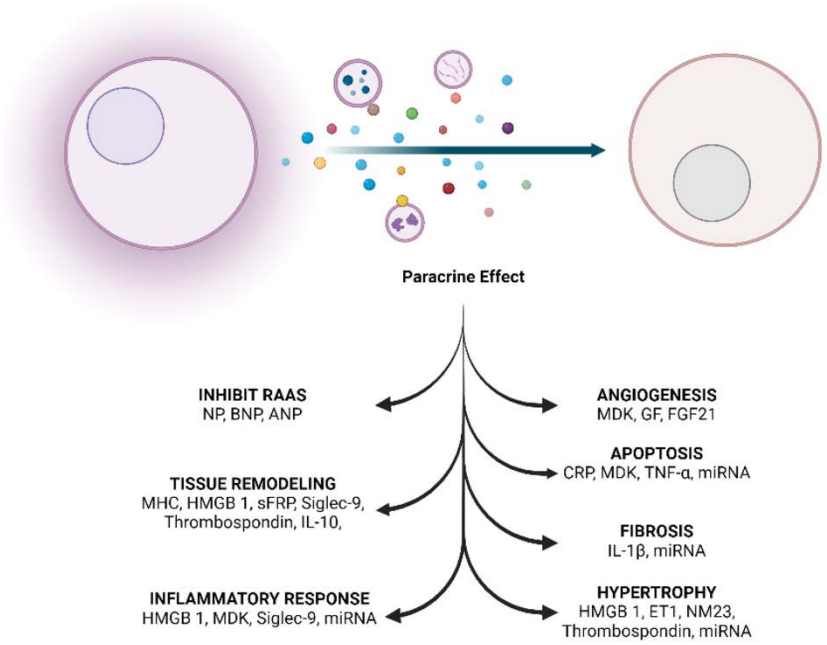

Figure 1. Biological roles of secretome and exosomes in cardiovascular disease progression. Cells express secretome, as a paracrine factor, in response to environmental changes or stress signaling. The factors are released in the form of proteins, microRNAs, and vesicles, including exosomes. The factors can be divided into different groups according to their roles. Created with Biorender.com premium license by Eka Ginanjar.

(natriuretic peptide (NP), B-type natriuretic peptide (BNP), and atrial natriuretic peptide (ANP)); angiogenesis (midkine (MDK), growth factor (GF), and fibroblast growth factor 21 (FGF21)); tissue remodeling (myosin heavy chain (MHC), high mobility box group box 1 (HMGB 1), secreted frizzled-related protein (sFRP), sialic acid binding immunoglobulin like lectin 9 (Siglec-9), Thrombospondin, and Interleukin-10 (IL-10)); apoptosis (C-reactive protein (CRP), MDK, tumor necrosis factor-alpha (TNF-α), and miRNA); fibrosis (Interleukin-1 Beta (IL-1β), and miRNA); inflammatory response (HMGB 1, MDK, Siglec-9, and miRNA); and hypertrophy (HMGB 1, Endothelin-1 (ET-1), Non-metastatic protein clone 23 (NM23), thrombospondin, and miRNA).[8]

Exosomes play a crucial role in controlling the progression of cardiovascular disease by facilitating the transportation and exchange of signaling chemicals. Exosomes also play a crucial role in facilitating communication between different cell types, both in pathological and healthy conditions. The specific miRNA cargo and rate of secretion may undergo modifications in response to pathological conditions or pharmaceutical intervention.[6] Cardiovascular exosomes have various effects on cells, including inducing pathology by transferring miRNA-21, promoting cardiac hypertrophy, and facilitating communication between cardiomyocytes and cardiac fibroblasts. These exosomes may also contribute to disease pathology and impede the effectiveness of heart regeneration treatments.[5]

The role of the MSC secretome and exosome has been studied for its benefits in pathological conditions such as cardiovascular disease (**Figure 2**). MSCs express the secretome in response to environmental changes or stress signaling. The product is released into the extracellular matrix (ECM) to create an environment that is favorable for cell regeneration. The products released include pro-angiogenic factors (vascular endothelial growth factor (VEGF), insulin-like growth factor-1 (IGF-1), and basic fibroblast

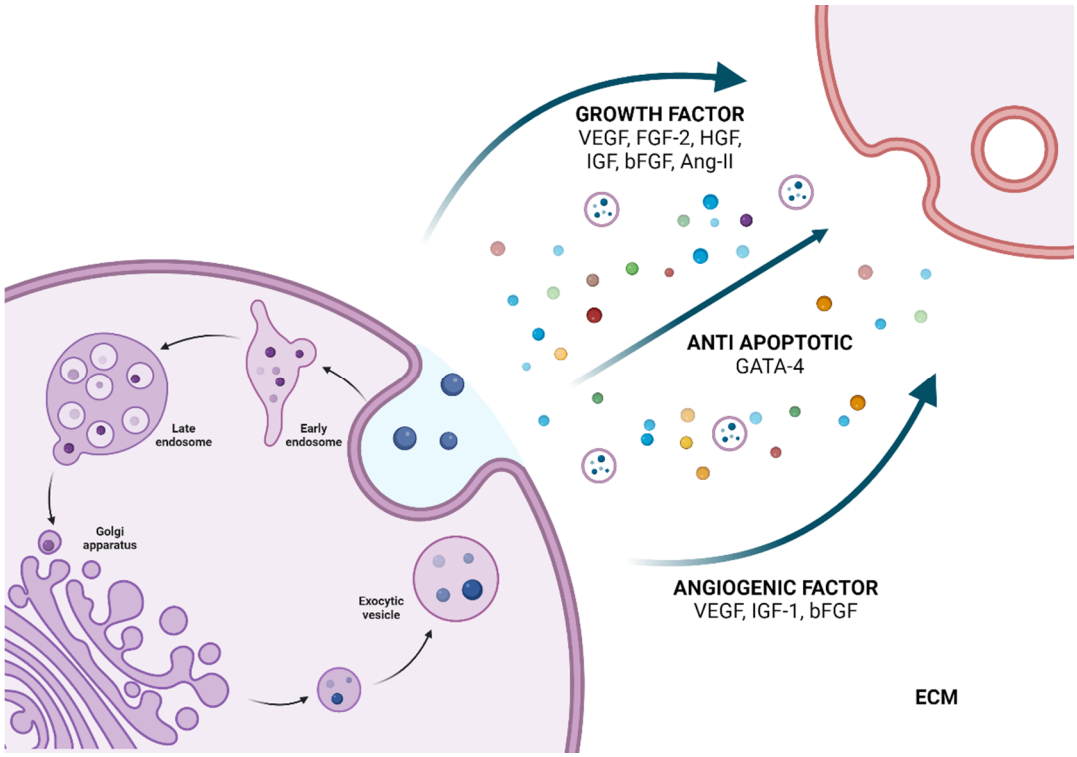

Figure 2. Biological roles of the MSC secretome and exosome in cardiovascular diseases. MSCs release the paracrine factor in response to environmental changes or stress, including proangiogenic, anti-apoptotic, and growth factors. Created with Biorender.com premium license by Eka Ginanjar.

growth factor (bFGF)), anti-apoptotic effect (GATA-4), and growth factors (VEGF, fibroblast growth factor-2 (FGF-2), hepatocyte growth factor (HGF), IGF, bFGF, and angiotensin-II (Ang-II)).[3]

Usefulness of Secretome and Exosomes in Cardiovascular Diseases

Biomarker

The usefulness of secretome and exosomes as biomarker s of pathological conditions of the heart is described in **Figure 2**. Secretome has been known for its use as a biomarker through the release of various proteins when pathological conditions occur in the heart, such as NP, BNP, ANP, FGF21, growth differentiation factors (GDF), MHC proteins, CRP, and troponins.[8] Before the detection of higher levels of conventional disease biomarkers like creatine kinase or troponin, an exosomal miRNA was identified, highlighting the potential of exosomes as a biomarker for cardiomyopathy. This suggests that exosomes could serve as an early, sensitive, and specific diagnostic tool for this condition.[9]

Delivery Vehicle for Therapies

Given their ability to traverse cellular membranes, exosomes have been investigated as a delivery vehicle for various therapies (**Figure 3**).[10–12] Exosomes contain many of the desirable characteristics of an ideal drug delivery system, as opposed to currently available delivery vehicles such as liposomes and polymeric nanoparticles. Exosomes can survive macrophages and circulate throughout the body due to their small size and biological origin. Exosomes have the capacity to avoid the endosomal pathway and lysosomal breakdown while still delivering cargo directly into the cytoplasm. Thus, transfection efficiency for molecules such as short interfering RNA (siRNA) can be enhanced.[11] Given their endogenous origin, surface composition, and high biocompatibility, EVs appeared to be less immunogenic. Another advantage of EVs lies in their potential capacity to transcend biological barriers—such as the blood-brain barrier, which is notoriously difficult to overcome with synthetic drug delivery vehicles.[12]

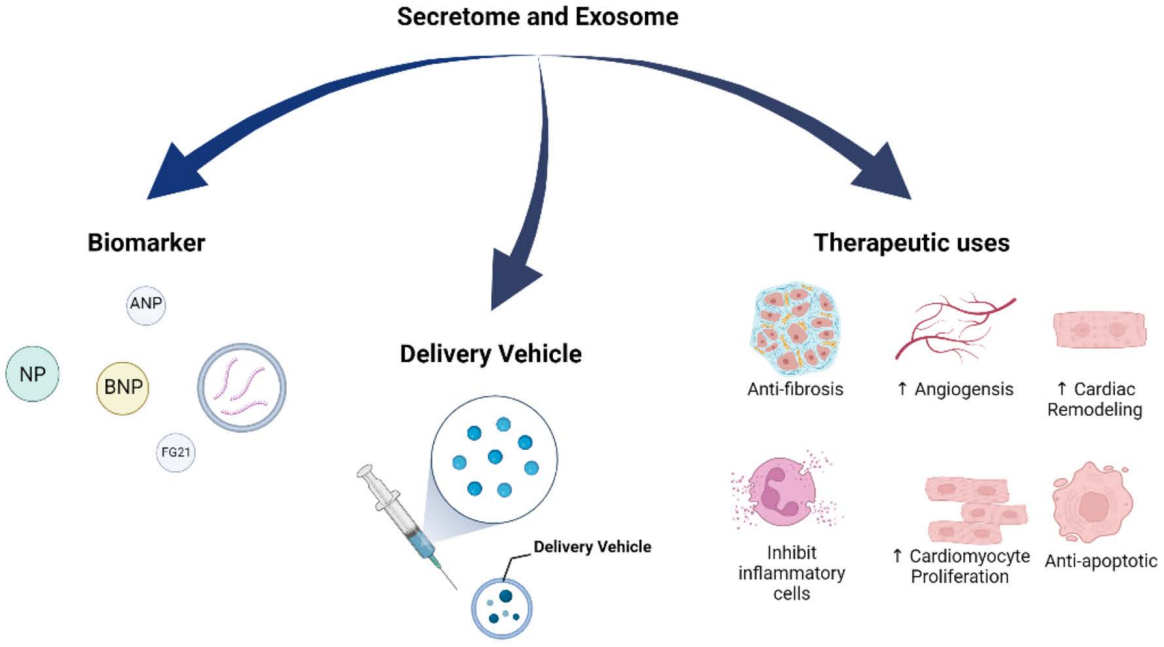

Figure 3. Usefulness of secretome and exosomes in cardiovascular diseases. Secretome and exosomes serve as biomarkers, therapeutic delivery vehicles, and provide therapeutic benefits such as promoting angiogenesis, enhancing cardiac remodelling, stimulating cardiomyocyte proliferation, exhibiting anti-apoptotic properties, inhibiting inflammatory cells, and providing anti-fibrotic effects. Created with Biorender.com premium license by Eka Ginanjar.

The use of exosomes as delivery vehicles has been widely determined to deliver therapeutic components in the form of small molecules, proteins, and nucleic acids. Exosomes are effective in delivering siRNA to the brain in mice. Exogenous siRNA was loaded into purified exosomes and intravenously administered. RVG-targeted exosomes transport GAPDH siRNA to neurons, microglia, and oligodendrocytes in the brain. The effectiveness of exosome-based siRNA delivery as a therapeutic approach was demonstrated by a strong knockdown in BACE1 mRNA (60%) and protein (62%).[10] In the cardiovascular field, studies have shown that a direct injection of hyaluronic acid (HA)–embedded EVs into infarcted myocardium improved angiogenesis, reduced apoptosis and fibrosis, and preserved cardiac function.[12]

Therapeutic uses of the MSC secretome in cardiovascular diseases

MSCs are known to produce a variety of immunomodulatory agents, as well as trophic factors that aid in repair and regeneration (Figure 3). The human MSC (hMSC) secretome comprises many molecules known to enhance cardiovascular healing, along with substances that adversely influence cardiomyocyte apoptosis, inflammation, and pathological remodeling. MSC secretome components provide a wide range of possible therapeutic mechanisms for cardiovascular repair, including tissue preservation (anti-apoptotic and promitotic), neovascularization, cardiac remodeling (ECM modification and reinforcement of infarct scar), anti-inflammatory responses (anti-fibrosis and inhibition of inflammatory cells), and endogenous regeneration (activation of cardiac-resident progenitor cells (CPC) and cardiac stem cells).[3]

In ischemic animal models, MSCs may facilitate neovascularization through paracrine signaling and exhibit anti-apoptotic, anti-inflammatory, and anti-fibrotic effects on cardiomyocytes and endothelial cells. MSCs have immunomodulatory effects by stimulating adjacent cells to produce cytokines, which may be advantageous in preventing excessive inflammation and pathological remodeling after cardiac ischemia. In inflammatory heart diseases, it has been observed that MSC-induced immunomodulation and anti-apoptosis of cardiomyocytes are likely mediated by paracrine effects.[3]

Therapeutic uses of the exosome in cardiovascular diseases

Exosomes are known to play a crucial role in controlling the progression of cardiovascular disease by facilitating the transportation and exchange of signaling chemicals (Figure 3). A study has discovered that cardiac fibroblasts release miRNA-enriched exosomes and identifies fibroblast-derived miR-21_3p as a paracrine signaling modulator of cardiomyocyte hypertrophy, which may serve as a therapeutic target.[13] Another study identifies upregulation of exosomes released induced by Ang II, which in turn activated Akt and mitogen-activated protein kinases (MAPKs) in cardiomyocytes. This results in increased Ang II production and cardiomyocytes' expression of its receptor, exacerbating Ang II–induced pathological cardiac hypertrophy.[14] A different study discovered that the therapeutic effects of remote ischemic conditioning (RIC) treatment for ventricular dysfunction following MI may be facilitated via exosome-mediated intercellular communication. The left ventricular ejection fraction (LVEF) of the RIC group was much higher than that of the untreated group, even though the severity of the MIs was comparable.[15]

In ischemic heart diseases, exosomes are found to have a cardioprotective effect against cardiac ischemia–reperfusion injury through the expression of heat shock protein (HSP) 70 that activates the toll-like receptor (TLR) 4 and many kinases, thereby initiating pro-survival signaling pathways in cardiomyocytes. TLR4 and traditional cardioprotective HSPs are involved in the process by which exosomes transport endogenous protective signals to the heart.[16]

The cardiosphere-derived cells (CDCs) CADUCEUS experiment found that CDC exosomes increase angiogenesis, prevent apoptosis, and encourage cardiomyocyte proliferation. The results show that exosomes are important mediators of CDC-induced regeneration and emphasize their potential use

as cell-free therapeutic possibilities.[17] Another recent study found that EVs secreted by MSCs may promote angiogenesis. This study demonstrates that intramyocardial injection of MSC-EVs significantly improves blood flow recovery, as evidenced by decreased infarct size and preserved cardiac systolic and diastolic performance using an acute myocardial infarction rat model.[18]

Several studies identify exosome cardioprotective effects with in vivo and in vitro models. Cardiac-resident progenitor cell–derived EVs, in which exosomes were a major component, decreased apoptosis in cardiomyocytes 48 hours after serum starvation. Furthermore, when injected into the myocardium in vivo, CPC-derived EVs increase blood vessel density, decrease cardiomyocyte apoptosis, and boost myocardial vitality.[19] CPC-derived exosomes are proven to prevent oxidative stress-induced apoptosis in cardiomyocytes in vitro via MiR-21 contained in the exosomes.[20] Exosomes originating from cardiospheres exhibit cardioprotective advantages in vitro by promoting angiogenesis, stimulating cardiomyocyte proliferation, and reducing apoptosis.[17] Cardiosphere-derived exosomes reduce myocardial infarct size in vivo by delivering miR-146a76 and activating protective antioxidant and pro-survival signaling pathways.[21] Exosomes produced from MSCs affect cardiomyocytes by reducing apoptosis and preserving mitochondrial membrane potential via the enrichment of miR-19a, and they influence endothelial cells by promoting angiogenesis and stimulating survival, proliferation, and tube formation.[22]

CONCLUSION

In conclusion, this literature review highlights the significant potential of cell-free therapy in the treatment of cardiovascular diseases (CVDs). Exosomes and secretome offer diverse functions, including intercellular communication, tissue repair, and immunomodulation. They have demonstrated potential in reducing the advancement of cardiovascular disease by inhibiting inflammation, promoting the growth of new blood vessels, and regulating crucial biological mechanisms. Both preclinical and clinical studies provide evidence of the effectiveness of exosome- and secretome-based treatments in addressing various CVDs. Further investigation is required to maximize the potential of secretome and exosome utilization as advanced cardiomyopathy therapy, both ischemic and nonischemic.

CONFLICT OF INTEREST

The author declares that he has no conflict of interest.

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