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# Advances in studies on exosomes and microvesicles as markers of cardiovascular disease

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**Abstract. – Cardiovascular diseases lead to one of the diseases with high mortality worldwide. Although considerable progress has been made in the diagnosis, treatment and prognosis of cardiovascular disease, there is still an urgent need for new diagnostic biomarkers and new treatments to reduce the incidence and mortality of the disease. In recent years, there has been increasing evidence that extracellular vesicles (EVs), especially exosomes and microvesicles (MVs) can be used as diagnostic biomarkers of protein and genetic information transmitted between adjacent and distant cells. This article summarizes various signal transmission pathways of exosomes and MVs in cardiovascular diseases, as well as their application and suggestions in the diagnosis of cardiovascular diseases.**

*Key Words:*
Cardiovascular disease, Exosomes, MVs, Markers.

## Introduction

Cardiovascular diseases (CVD) are the world's largest cause of death. It is estimated to cause more than 17.9 million deaths annually, accounting for 31 percent of global deaths. CVD have huge health and economic burden[1]. CVD are complex and can cause heart and vascular-related diseases at the same time, such as coronary heart disease, myocardial infarction (MI), myocarditis, valve disease or heart failure (HF), etc[2]. The interaction and influence of various types of cells in the heart, including cardiac cells, smooth muscle cells, endothelial cells and fibroblasts, maintain their basic physiological functions, and can also respond to these pathological changes through a series of intercellular signaling and intercellular matrix changes, a process collectively referred to as heart remodeling[3-5]. New research[6-8] shows that this important self-regulating function of heart remodeling is to use EVs, or more accurately, exosomes and MVs. EVs can protect nucleic acids and other biological macromolecules from *in vivo* degradation and are delivered to receptor cells in a targeted non-immune activation manner. They also have the ability to carry genetic material, including RNA, DNA, metabolites, lipids, cytosolic and cell-surface proteins[9-11]. Exosomes and MVs are key factors in regulating cardiovascular function and play an important role in almost all aspects of cardiovascular biology.

### Overview of Exosomes and MVs

EVs are lipid binding vesicles secreted by cells outside the cell, about 50 nm to 2 μm in size[12]. The three main types of EVs are exsosomes, MVs and apoptotic bodies (ApoBDs), can be distinguished according to their formation pathway, size and function[13,14]. When multivesicular bodies fuse with the plasma membrane in the process of height regulation, a homogenous population of exovesicles is released from the cell, of which exosomes attract the most attention[15]. The cells also produced a heterogeneous population of exovesicles up to 2 μm in diameter, called MVs, which is formed through the budding and shedding of the cell membranes, a process involving direct dynamic interaction between phospholipid redistribution and cytoskeletal protein contraction[16,17]. Cells undergoing apoptosis usually release outer vesicles with a diameter of 1-5 μm from their breakdown products, known as apoptosis[18]. EV research is aimed at isolation methods, classification of EVs, and find out different functions in disease progress and therapy[19,20]. The main concerns in this review are two major categories, exosomes and MVs.

Exosomes are first observed from nanovesicles secreted by mature reticulated erythrocytes by Pan and Johnstone in 1983[21]. More than a decade later, Raposo et al (1996)[22] and Zitvogel et al (1998)[23] proved that B cells and dendritic cells release the vesicles in a similar way, respectively.



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In subsequent years, many studies also showed several cell types from other sources, such as platelets, mast cells, neurons, oligodendrocytes, Schwann cells and intestinal epithelial cells, have been shown to release exosomes[24,25]. In addition, exosomes are also found in all biological fluids[14]. Comparing the formation mechanism of the external urinary body and other types of membrane vesicles, the formation of intracellular multivesicular bodies (MVBs) containing intraluminal vesicles (ILVs). Many researchers believe that exosomes have the potential to be applied clinically or used as a biomarker or treatment tool for related diseases[26].

MVs have long been regarded as "cell fragments", but that view is changing rapidly. Wolf first described the release of MVs in 1967[27]. This kind of vesicles (0.1-2 μm) are formed by protruding or buds of the plasma membrane. They can be activated by calcinase, and calcium internal flow and cytoskeleton recombination are released from the surface of the cell membrane to the extracellular environment[28]. In the past few decades, there has been increasing evidence that MVs are fragments of small membranes that fall off eucaryotic cells during activation or apoptosis, which play an important role in the pathogenesis and subsequent progression of various human diseases[29]. Changes in MVs have been detected in various body fluids of patients with multiple diseases[30,31]. MVs vary greatly due to their different sources and have heterogeneity, but all have spherical and lipid bilayer structures[32]. In fact, some of them may contain specific biomarkers from the surface of their parent cells, so specific biomarkers on the detection of vesicles can be used to identify their cell origins[33]. MVs induced under different stimulation conditions may carry different ingredients and will become an important medium for intercellular communication[34]. Therefore, the release of MV provides a new idea for the study of disease development or pathological state[35].

## Information Transfer of Exosomes and MVs in Cardiovascular Disease

### *Information Transmission of Exosomes*

Exosomes' natural constituents may play a role in minimizing adverse reactions and in enhanced bioavailability. Exosomes are highly enriched with protein involved in membrane transport and fusion, such as quadrangle transmembrane proteins (CD9, CD63, CD81, CD82), which are involved in cell penetration, invasion, and fusion[36]. There is also a link between part protein and receptor-dependent signalling pathways carried by exosomes, which may mediate activation of immunosuppression and anti-inflammatory pathways[37]. The exosomes are derived from stem cell in cardiovascular protection has emerged as a potential therapeutic approach[38]. In addition, the external membrane contains heat shock proteins such as HSP70 and HSP60, which participate in the binding and presentation of antigens as part of the stress response and are responsible for membrane transport and protein fusion[39]. HSP70 binds to TLR4 on myocardial cells to activate downstream phosphorylation of ERK1/2, p38MAPK and small thermal shock protein HSP27, ultimately achieving the purpose of protecting myocardial cells[37]. The role of plasma exosomes in ischemia pretreatment (RIC) was verified by differential hypercentrifugation from normal rats or healthy human bodies. The experimental results show that these plasma exosomes have a cardiac protective effect. This mechanism of action is still reflected in rat heart perfusion and primary myocardial cell models. Although the exosomes are not ingested by myocardial cells, they activate the corresponding signalling pathway by acting on myocardial membrane protein and continuously send signals to the heart, thus regulating and protecting the state of myocardial cells[40].

In 2007, Valadi et al[41] first described the presence of mRNA and miRNA in exosomes, who participate in the transfer of functional RNA between cells and can play a role in receptor cells. The RNA fragments contained in the exosomes vary according to the origin of cell type or the occurrence and development of disease. In recent in-depth sequencing studies, exosomes actually contain diversified RNAs, including hundreds of non-coding RNAs and special Y-RNA fragments[42-44]. Y-RNA fragments (EV-YF1) actually contain rich snRNAs, which are carried by exosomes to protect the heart after cardiac ischemia or reperfusion[45]. At present, in the field of cardiovascular surgery, the importance of miRNA regulation in many physiological or pathological processes is recognized[46]. Blood exosomes were isolated using differential speeding centrifuges, and there were about 1010 exosomes /mL in the blood providing receptors or ligands of different miRNAs for media transmission of heart signals[47]. Exosomes secreted by cardiac fibroblasts are rich in miR-21-3p, which causes myocardial

cell hypertrophy during normal myocardial cells ingestion of these exosomes[48]. Through shear stimulation, the functionally active miR-143/145 in exosomes is transported to smooth muscle cells, enabling smooth muscle cells to express the resist atherosclerosis gene. Further experiments in animals that act on ApoE(-/-) mice with the corresponding exosomes in the body, and the results show that the exosomes containing active miRNA do relieve atherosclerosis in the aorta of these mice[49]. All of the above clues indicate that RNA molecules are not randomly loaded into exosomes but have a mechanism that actively regulates specific RNAs into exosomes and participates in the occurrence and development of diseases.

### Information Transmission of MVs

Increasing evidence shows that MVs (MVs) can contain not only membrane proteins and lipids from the cell surface, but also nucleic acids (DNA and RNA), including mRNAs, microRNAs (miRNAs), small interfering RNAs (siRNAs) and long non-coding RNAs (lncRNAs) from the intracellular environment, these nucleic acid substances can participate in the "re-editing" of recipient cells[50]. Extracellular miRNAs packaged by MVs have been shown to play an important role in inflammatory, cardiovascular, and metabolic diseases[51,52]. Circulating MVs, as transportation carriers of a variety of miRNAs, participate in the basic signal conduction process of CVD[53]. For example, human-derived THP-1 cells in inflammatory factor therapy contain miR-150, while miR-150 targets c-Myb in endothelial cell migration[54]. In addition, MVs carrying miR-126 promote angiogenesis by activating the PI3K/Akt signaling pathway[55]. MVs from endothelial progenitor cells are related to specific mRNAs and can stimulate angiogenesis in endothelial cells through the phosphatidylinol 3kinase/protein kinase B signalling pathway. These studies suggest that RNAs carried in MVs might play an important role in transferring gene regulation functions.

When the MVs are in contact with the specific receptor, they are activated by endocytosis by the receptor cell or by fusion of the receptor cell membrane[56]. At this time, MVs containing bioactive components may be involved in and mediate a variety of cardiometabolic diseases. Exposure of phosphatinine serine and thrombin during MV formation can induce vascular inflammation and the formation of venous embolism, thus increasing the probability of cardiovascular disease and thrombosis formation[56,57]. In addition, recent studies have shown that MVs carry active decombin and metalloproteinase domain proteins (ADAM) in intralumbar vertebral thrombosis near abdominal aortic aneurysms (AAA), and may therefore be related to the degradation of the extracellular matrix of the aortic wall and the development of human AAA[58]. MVs secreted by activated platelets in tumor cells were released into the bloodstream[59], and finally platelet residues and MVs were found in the angiogenesis site. Moreover, MV can play an important role in angiogenesis by transferring a series of proangiogenic factors, including growth factors such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and platelet-derivated growth factor (PDGF)[60]. These released MVs not only enter the circulation system as ordinary vesicles, but also target adjacent or distant receptor cells, fuse with the target cell plasma membrane, and eventually enter the cytoplasm or nucleus as carriers, affecting the function and phenotype of the receptor cell[61]. It can be seen that the role of these special MVs has changed from biological markers to related effectors of intercellular signal transmission.

## The Role of Exosomes and MVs in CVD

### Role of Exosomes in the Treatment of CVD

At the early stage of exosomes discovery, researchers focused more on the important role of exosomes in the mechanism of tumorigenesis and development[62]. There is increasing evidence that the exosomes are involved in the regulation of cancer cells and normal cells, and play an important role in intercellular information exchange, such as carrying multiple cytokines (interleukin, tumor necrosis factors, etc.) into the cell microenvironment, thus promoting angiogenerative tumors and tumor cell metastasis[63,64]. In recent years, with the deepening of exosome research, it has been confirmed that exosomes extensively participate in the process of intercellular information exchange in the physiological and pathological process of the cardiovascular system, and plays an important role in the occurrence, development and treatment of CVD.

The heart is a terminally differentiated organ, meaning that there is very little division of cardiomyocytes after injury, and instead com-

pensative regulation is achieved through cardiac hypertrophy. Scholars have hoped to renew myocardial cells through stem cell therapy for many years, but the results of this method are largely disappointing. The latest experimental results show that some improvements in cardiac function can be observed after stem cell treatment, but it is interesting that the benefits of similar levels of myocardial cells can also be observed after injecting the cultured stem cell medium[65]. It is proposed that mature stem cells release growth factors and chemokines that promote heart repair through paracrine to improve the survival and function of myocardial cells[66]. In 2010, exosomes purified from mesenchymal stem cells (ESC-MSC) derived from human embryonic stem cells (ESC) through HPLC method can protect myocardial cells and the heart *in vitro* and *in vivo* experiments[67]. The results of another study[68] showed that Akt-mediated increased GSK-3β activity with the corresponding exosome acting in the experiment for 1 hour, which improved the contractile function of the heart after 28 days.

Some scholars[69,70] tried to use the overexpressed GATA-4 carried by the exosomes secreted by MSC cells and injected the exosomes into the heart of rats before coronary ligation, and the results showed that the infarction area decreased and improved cardiac contraction function. In addition to the exosomes secreted by MSC for the benefit of cardiovascular action, exosomes from mouse-derived cardiac progenitors (CPC), embryonic stem cells (ESCs) and induced pluripotent stem cells (iSPCs) are also associated with cardiac protection[71]. Further studies have found that the exosomes isolated from CPCs of the appendage implants of patients undergoing heart valve surgery and injected into the heart of rats with coronary artery ligation, the results showed a decrease in apoptosis and scar area of cardiomyocytes, an increase in the number of living cells and increased blood vessel density in the infarction area[72]. The results of this series of studies show that exosome does have a functional effect on a variety of CVD and has the potential to treat or improve cardiovascular conditions

## Application of MVs in the Diagnosis and Prognosis of CVD

MVs are mass membrane vesicles produced by almost all eucaryotic cells during activation or apoptosis, and are released from the surface of the cell membrane to the extracellular environment by sprouting[73]. MVs, as an extracellular carrier, transports biologically active molecules from parent cells to receptor target cells, which is expected to become a new medium for intercellular communication[74]. This kind of vesicles contain various components from their parent cells, so they can perform various functions of intercellular communication, signal transduction and immunomodulation. Martinez et al[75] proved that circulating MVs can serve as biomarkers for monitoring various heart metabolic diseases. Heart failure (HF) results from the cardiac remodeling post MI and can be caused by stress through various adverse conditions. There is increasing evidence that HF and cardiovascular complications are primarily due to endothelial dysfunction[76,77]. Nozaki et al[78] recruited 169 HF patients to continuously detect circulating MV levels, proving that increased MVs can provide valuable information for HF patients with future cardiovascular conditions. In addition, quantitative circulating MVs in plasma can be used as biomarkers for the diagnosis and treatment of perinatal cardiomyopathy[79] or vascular sclerotic cardiomyopathy[80].

However, MVs are not necessarily detrimental in the development of CVD, may have anticoagulant properties by carrying thrombomodulin[81]. In addition, gene therapy and specific drug delivery through package and release of MVs in the future[82]. The advantages of applying MVs in therapeutic delivery include decreased toxicity or immunogenicity and increased stability of intracellular environment[83].

CAD, featured by stenosis or obstruction of coronary artery, leads to the occurrence of myocardial ischemia or even infarction. Wallace et al[50] have shown elevated MVs levels in patients with CAD as compared to healthy controls. Cholesterol-lowering statins have been proved to reduce the levels of circulating MVs derived from leukocytes, platelets, and endothelial cells[84], as well as other heart protection drugs, including calcium blockers[85], aspirin[86] and clopidogrel[87]. Although the potential mechanism has yet to be clarified, after improving the overall cardiovascular state, the level of MVs in the blood has also changed accordingly. Changes in the level of MVs allowing for a multicomponent diagnostic window into disease detection and monitoring.

## Summary and Outlook

It is now understandable that exosomes and MVs play a key role in cardiovascular health and disease. Over the past few years, researchers

have begun to recognize the biological processes of exosomes and MVs as vectors for intercellular transfer genetic factors and biological signals. These EVs are believed to expand the ability of cells to transmit beneficial and harmful molecules throughout the organism. Due to the ability of the exosomes and MVs to carry a variety of information, they are now considered to have therapeutic molecules that enter previously inaccessible heart areas as ideal vectors. Research on exosomes and MVs, including the discovery and updating of mechanisms and effects, will obtain targeted treatments to solve various CVD.

### Conflict of Interest

The Authors declare that they have no conflict of interests.

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