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Functional roles of exosomes in cardiovascular disorders: a systematic review

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Abstract. – Cardiovascular diseases are major causes of people death associated with high mortality and disability. Exosomes are nano-sized extracellular vesicles containing protein, lipid, transcription factors, mRNAs, non-coding RNA (ncRNA) and nucleic acid contents, which are critical players of intercellular communication via long-range signals or cell-to-cell contact. The emergence of exosomes provides favorable strategies for the diagnosis and treatment of cardiovascular diseases. Exosomes-based molecular mechanisms are important for developing novel therapeutic approaches for cardiovascular events. In this review, we will (1) provide insights into the detrimental and beneficial effects of exosomes on cardiovascular physiology, (2) summarize the underlying biological mechanisms of the exosome in cardiovascular events, (3) investigate the therapeutic value of exosomes for cardiovascular disorders.

Key Words:
Exosomes, Cardiovascular diseases, Endothelial cell.

Introduction

The prevalence of cardiovascular diseases is markedly increased in low- and middle-income countries for decades[1,2]. Over 4.3 million deaths are induced by cardiovascular diseases every year in Europe, which brings a considerable burden on the economy of European Union[3]. The Centers for Disease Control and Prevention have announced that $444 billion may be used for the treatment of cardiovascular diseases in 2010, and the costs will be enhanced with the increase of life expectancy[4]. Development of novel diagnostic or therapeutic strategies may provide multiple opportunities for reduction in mortality of cardiovascular diseases.

Exosomes have obtained substantially attention due to their potential therapeutic applications[5]. A wide range of researches has investigated the roles of exosomes in cancers[6,7], neurologic disorder[8], endocrine system diseases[9], autoimmune diseases[10] and cardiovascular diseases[11]. Exosomes are involved in various biological activities including cell proliferation and differentiation[12], inflammation[13], senescence[14], angiogenesis[15], stress response[16] and cardiovascular remodeling[17]. Exosomes-mediated intercellular communication plays a fundamental role in vascular integrity and cardiovascular diseases[18].

Exosomes are associated with many cardiovascular pathologies such as cardiac hypertrophy[19], atherogenesis[20], heart failure[21], hypertension[22] and diabetic cardiomyopathy[23]. Mounting evidence has shown that exosomes may transfer non-coding RNA (ncRNA) including miRNA and lncRNA to recipient cells, thus leading to the changes in protein expressions and phenotypes of recipient cells[24,25]. Exosomes are recently used as disease biomarkers[26], therapeutic targets[27], agents for drug delivery[28] and biomedical applications[29]. The following review will summarize the intercellular signaling, possible mechanisms, prognostic, diagnostic and therapeutic roles of exosomes and exosomal ncRNAs in cardiovascular diseases.

Biogenesis and Secretion of Exosome

Cell to cell communication between cardiovascular cells is a complex process that exerts a requisite role in cardiovascular biology[30,31]. Accumulating evidence establishes that exosomes are intercellular communication messengers[32,33]. The exosomes were firstly identified during the research on the formation of vesicle in 1987[34]. Exosomes are known to be one of the subtypes of membrane vesicles, whose sizes are ranging from 30 to 100 nm[35]. Exosomes are distinguished from apoptotic bodies and microvesicles due to their unique qualities[36].

It has been demonstrated that microvesicles are released from direct outward blebs of plasma. However, exosomes are produced by en-

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dosomal network[37]. The inward budding of cell membrane ligands leads to the fusion of small vesicles and early endosomes. The extracellular membrane ligands are internalized to surfaces of these small vesicles during this process. The second inward invagination of the endocytic vesicles membrane creates various intraluminal vesicles (late endosomes). The deposition of late endosomes is defined as multivesicular bodies. The multivesicular bodies are then fused into the cell membrane, following by release of intraluminal vesicles through an exocytotic way. The released intraluminal vesicles are referred to as exosomes. A wide coverage of cargos such as proteins, enzymes, ncRNA, mRNA, and molecules are presented within exosomes[23,38].

The constitutive or inducible pathways are responsible for the release of exosomes. In the literature, certain RAB GTPases[39-41], WNT5A[42], heterotrimeric G-protein[43], glycosphingolipids and flotillins[44] can modulate the constitutive secretion of exosomes. Numerous factors including calcium release-dependent mechanism[45], heat shock[46], hypoxia[47], thrombin[48], DNA damage[49], lipopolysaccharide[50,51] participate in the secretion of exosomes.

Characterization of Exosomes

Electron microscopy is a critical step in the characterization of exosomes. Transmission electron microscopy can clearly capture the photographs of exosomes with the aid of uranyl acetate and methylcellulose. Exosomes are observed as double-membrane bound vesicles under electron microscopy[52]. The "cup-shaped" morphology of exosomes can be distinguished on electron micrograph[53]. Furthermore, standard preparation techniques are applied to identify exosomes on tissues using electron micrographs[54].

It is noted that exosomes are generated from endosomal pathways, antibodies against endosomal markers may be employed to characterize the exosomes. Tetraspanins (CD9, CD63, and CD81), and phosphatidylserine are abundantly expressed within exosomes[55]. Combinations of antibodies and electron micrograph methods are recommended to obtain accurate confirmation of exosomes.

Flow cytometry is applied to examine fluorophores-tagged exosomes, but it is unable to quantify the exosome numbers due to swarming effects[56,57]. The exosomes are marked by membrane-binding dye such as PKH67, which can be seen under fluorescence and confocal microscopy. Such technics could determine whether marked exosomes are absorbed into recipient cells[58]. Moreover, small-angle X-ray scattering[59], resistive pulse sensing[60], and Raman microspectroscopy[61] are novel methods for detection of exosomes.

Cellular Communication Functions

Cell junctions, adhesion contacts, and soluble factors are classical molecules, and they act on targeted cells in an endocrine manner[62]. Extracellular vesicles transfer the various proteins, lipids, and nucleic acids into recipient cells, thus causing changes in intracellular signaling of recipient cells[52]. A growing body of evidence indicates that the proteins, mRNA, miRNA and lnc RNA within exosomes are inserted into recipient cells, thus inducing transient or persistent phenotypic changes in recipient cells[63]. It is interesting that the small RNAs in the exosomes are surrounded by lipids or lipoprotein complexes, which may protect them from degradation during the transport processes[64]. The exosomes are involved in various physiological or pathological processes such as regulation of tumor growth, cytokine production or cardiovascular disorders[9,65,66].

Biomarkers, Diagnosis, and Therapy of Exosomes

With the deepening of research on exosomes, the exosomes may be served as valuable biomarkers, diagnostic, prognostic and therapeutic tools for cardiovascular diseases[67,68]. MiR-133a-containing exosomes are a useful biomarker for myocardial damage or cardiomyocyte death[69]. It is revealed that the levels of miR-15b, miR-34a, and miR-636 within urinary exosomes are enhanced in patients with type 2 diabetic kidney disease, and these urinary exosomal miRs are treated as a novel diagnostic panel for diabetic kidney disease[70]. Bioinformatics analysis establishes that urinary exosomal miR-133b, miR-342 and miR-30a are closely associated with systolic-diastolic blood pressure, serum creatinine, urinary albumin creatinine ratio and glomerular filtration rate in diabetic nephropathy[71].

The biomolecules and bioactive molecules such as proteins, enzymes, growth factors, mRNA, DNA, and ncRNAs in exosomes facilitate the exosomes to be a therapeutic tool in many diseases[72]. In addition, exosomes are chemically modified to be a delivery tool for transferring the specific bioactive molecules into certain cell types[73]. The exosomes-carrying tumor antigens

induce T-cell lymphocyte responses and inhibit tumor growth[74]. The potential roles of exosomes in cardiovascular diseases are intensively investigated in recent years. The exosomes derived from dendritic cells stimulate CD4(+) T lymphocytes activation to improve cardiac function after myocardial infarction in mice[75]. The cardiomyocyte-released exosomes transfer glucose transport to endothelial cells, thus inducing glucose uptake, glycolytic activity, and pyruvate production in endothelium[76]. Mesenchymal stem cells (MSCs) overexpressing GATA-4 releases exosomes containing a reservoir of anti-apoptotic microRNAs to rat neonatal cardiomyocytes, contributing to cardiomyocytes survival under hypoxic environment[77].

To date, the possible roles of exosomes in cardiovascular diseases have not yet been fully elucidated in the clinical practice. More and more studies should be conducted to examine diagnostic, prognostic value and functional roles of exosomes content in cardiovascular diseases.

Exosomes and Diabetes Mellitus

Diabetes mellitus is a widely prevalent disorder around the world[78,79]. The exosomes are closely associated with diabetes in diabetic patients or diabetes models[80-88]. Plasma exosomal miR-326 levels are up-regulated, but let-7a and let-7f levels are down-regulated in diabetic patients, the levels of let-7a and let-7f in plasma exosomes are significantly increased after antidiabetic treatment[81]. The cardiomyocyte-derived exosomes from diabetic rats inhibit the proliferation and migration of endothelial cells, but the exosomes from normal rats accelerate the proliferation and migration of endothelial cells[88]. It has been recently reported that the cardiomyocytes-derived exosomes contribute to increases in glucose uptake, glycolysis in endothelial cells under glucose deprivation conditions[76]. The cardiomyocytes transfer the exosomal miR-320 into endothelial cells to mediate angiogenesis in type 2 diabetic rats[83]. The exosomes from bone marrow-derived mesenchymal stem cells are transferred into damaged neurons and astrocytes, which significantly improved cognitive impairment in diabetic mice[89]. A large prospective study has concluded that exosomes containing miR-126 have a predictive value for cardiovascular events in patients with stable coronary artery disease[90]. The endothelial cells-derived exosomes promote vascular endothelial repair via transferring the miR-126 into recipient cells, which is disrupted under hyperglycemic conditions[91]. The miRNA-enriched exosomes from fibrocytes accelerate wound healing in diabetic mice[92]. The exosomes are ideal candidates for illumination of diabetic pathophysiology, and may provide novel therapeutic approaches for diabetes.

Exosomes and Myocardial Infarction

Myocardial infarction is reflected by occlusion of coronary vessels and cardiac cell death[93,94]. The molecule mechanisms for cardiac rehabilitate response to myocardial infarction are not fully explained[95]. Coronary bypass surgery and balloon dilatation of coronary vessels are usually used to alleviate cardiac impairment in the acute phase of myocardial infarction[96]. Novel strategies or technics are urgent to be developed for improvement of cardiac tissue repair. The exosomes are critically involved in the proliferation and apoptosis of targeted cells[97]. A plethora of researches has identified the roles of exosomes in cardiovascular diseases[98-100]. The exosomes are essential for local and distant microcommunication with recipient cells in myocardial infarction[12,101]. The cardiac progenitor cells[102] or embryonic stem cells-releases exosomes[103] regulate cardiac regeneration and cardiac remodeling during the myocardial infarction.

Mesenchymal stem cells are able to deliver miR-22-shutting exosomes into neonatal rat ventricle cardiomyocytes, leading to reduced apoptosis of cardiomyocytes[104]. Cardiac progenitor cells contribute to decreased cardiac fibrosis, cardiomyocyte apoptosis, and increased angiogenesis or cardiac output after myocardial infarction via transferring antifibrotic miRNAs-enriched exosomes to fibroblasts under hypoxia[32,102]. The cardiosphere-released exosomes stimulate the proliferation and angiogenesis of cardiomyocytes[105]. The mesenchymal stem cell-derived exosomes preserve cardiac function, and relieve infarct size in ischemia reperfusion injury mode[106]. Intravenous administration of mesenchymal stem cells-derived exosomes decreases the infarct size by 45% and depresses systemic inflammation in ischemia-reperfusion model[107]. The exosomes from healthy controls exert a protective role in ischemic myocardium via delivering endogenous protective signals including cardio-protective heat shock protein 70[108]. Direct intramyocardial transplantation of exosomes from GATA-4 overexpressed mesenchymal stem cells obviously improve cardiac contractile function and alleviate infarct size in the rat heart[77]. These studies sug-

gest that exosomes from stem cells are believed to play protective roles in cardiac remodeling during the myocardial infarction.

Exosomes and Coronary Artery Disease

Atherosclerotic lesions are closely associated with endothelial cell activation, inflammation, formation of foam cells and phenotype transformation of VSMCs[109,110]. In primary rat aortic endothelial cells, the heat shock protein-70-carrying exosomes are increased in response to homocysteine and ox-LDL stimulation[111]. Heat shock protein-70 mediated proinflammatory genes contribute to monocyte adhesion in endothelial cells[112]. The heat shock protein-70-enriching exosomes may be responsible for subendothelial migration of monocytes in atherosclerosis. The activated macrophages secrete miR-223-containg exosomes to evoke an inflammatory response in atherosclerosis[113]. It has been shown that exosomes from atherosclerotic plaques are a stimulator for the adhesion molecule expressions, and inflammatory endothelial cells, which may be responsible for the plaque development[114]. The exosomes containing miR-143/145 are increased in human umbilical vein endothelial cells exposure to shear stress through modulation of shear-responsive transcription factor KLF2[115-117]. Cardiomyocytes and endothelial cells can communicate via exosomes-mediated exchanges[118,119]. Endothelial cells release miR-146a-bearing exosomes to cardiomyocytes, which downregulates the interleukin-1 receptor-associated kinase 1 and receptor tyrosine-protein kinase ERBB4 levels in cardiomyocytes[118,120].

Activated platelets-derived exosomes carry CD40 ligand to regulate the differentiation of antigen-presenting cells including monocyte-derived dendritic cells[121]. However, stored platelets-associated exosomes retard the differentiation from monocytes to macrophage and dendritic cell maturation[122]. It is seen that platelet-released exosomes may exert different effects on inflammation response. Also, the platelet-derived exosomes may participate in atherogenesis via hyperplasia of vascular smooth muscle cells[123] and proinflammatory activation of endothelial cells[124]. The monocytes-generated exosomes promote atherogenesis associated with activation of macrophages and endothelial cells[125]. The monocytes-derived exosomes are suggested to stimulate nitrosative stress in human endothelial cells[126].

Conclusions

In recent years, the exosomes are novel approaches or strategies for characterizing the communications between living cells. The functional roles of exosomes in cardiovascular disorders are summarized in Figure 1. The exosomes are taken as possible candidates for intercellular and tissue-level communication. Importantly, the exosomes-containing various proteins and RNA messages may be secreted to recipient cells, which modulates the targeted gene expressions in recipient cells. Furthermore, the epigenetic mechanisms such as histone modifications, DNA methylation, and non-coding RNA expressions play pivotal roles in various biological effects in cardiovascular diseases. It may be speculated that exosomes may carry epigenetic modulator to induce functional changes in recipient cells. It is interesting that exosomes from different cells may exhibit protective or destructive roles in cardiovascular diseases. The advanced technics to modify or load thera-

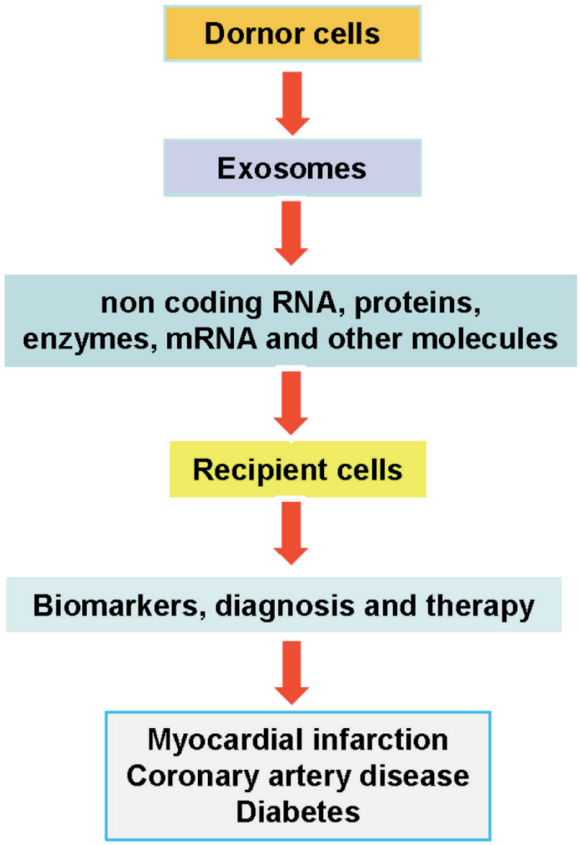

Figure 1. Functional roles of exosomes in cardiovascular disorders.

peutics into exosomes can be developed and standardized in a future study. It is undeniable that the unique opportunities and new challenges for characterization of exosomes as clinical biomarkers, diagnosis and prognosis factors in cardiovascular diseases are still on fire.

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Conflict of Interest
The Authors declare that they have no conflict of interests.

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