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Review article

Exosomes as agents of change in the cardiovascular system

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ABSTRACT

Exosomes have an evolving role in paracrine and autocrine signaling, which is enhanced because these lipid vesicles are quite stable and can deliver miRNA, DNA, protein and other molecules to cells throughout the body. Most cell types release exosomes, and exosomes are found in all biological fluids, making them accessible biomarkers. Significantly, exosomes can carry a biologically potent cargo, which can alter the phenotype of recipient cells. In the cardiovascular system exosomes have been primarily studied for their role in mediating the beneficial effects of mesenchymal stem cells after myocardial injury. Exosomes released by cardiac cells in disease states, such as myocardial ischemia, can potentially have important pathophysiologic effects on other cardiac cells as well as on distant organs.

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Abbreviations: CDC, cardiosphere-derived cell; CF, cardiac fibroblast; CPC, cardiac progenitor cell; CSC, cardiac stem cell; ESC, embryonic stem cell; ESCRT, endosomal sorting complexes required for transport; HSP, heat shock protein; ILV, intraluminal vesicle; MHC, major histocompatibility complex; miR, microRNA; MSC, mesenchymal stem cell; MVB, multivesicular body; N-Smase2, neutral sphingomyelinase2; RIC, remote ischemic conditioning; ROS, reactive oxygen species; Vps, vacuolar protein sorting-associated protein.

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1. Introduction

Exosomes are a subset of extracellular microvesicles (Table 1) first identified as part of reticulocyte maturation, where exosomes are a vehicle for removing unneeded proteins and membrane, as the reticulocyte transforms into a mature erythrocyte [1,2]. Electron micrograph studies of reticulocyte externalization of the transferrin receptor demonstrated the presence of small lipid vesicles, which were termed exosomes. Exosomes are the product of a series of steps involving the multivesicular body (MVB). MVBs form in cells by invagination of the cell membrane. Subsequently, exosomes are formed by an invagination of the MVB's, resulting in preservation of the original cell membrane orientation in the intraluminal vesicle (ILV) membranes. MVBs either fuse with the lysosome for protein degradation or with the plasma membrane, releasing ILVs into the extracellular environment as 30–100 nm microvesicles, exosomes [3] (Fig. 1). The regulation of this switch between the lysosome *vs.* release into the extracellular space remains undefined. Recently, exosomes have been identified as a mechanism of intercellular signaling through the delivery of proteins and nucleotides, which alter recipient cells. Exosomes were first identified to have a pathological role by Skog who found that glioblastoma tumor cells release exosomes, which can be taken up by normal neural cells, changing their phenotype to a more cancerous one [4]. Glioblastoma exosomes transferred mRNA to the normal neural cells, and this mRNA was subsequently translated [4]. Furthermore, proteomic analysis of glioblastoma exosomes, found they were enriched in angiogenic proteins, and after uptake they were able to stimulate endothelial cell tubule formation. Another example of potential exosome mediated intercellular signaling involves viral transmission. Viral infection is associated with increased exosome production, and it has been postulated that exosomes could mediate immune evasion by transmitting virus particles between cells [5,6]. Exosomes have been investigated in many biological fluids, and found to serve a number of roles from pathological to protective.

1.1. Pathways to exosome generation

The MVBs are late endosomes that mature from invaginations of the cellular plasma membrane. The formation and packaging of ILVs is still not fully understood, as exosomes do not contain random cytosolic proteins, but are enriched in certain proteins and nucleic acids relative to their parent cells [4,7–9]. One mechanism of exosome packing is through the endosomal sorting complexes required for transport (ESCRT) machinery (Fig. 2). ESCRT-0 is responsible for binding ubiquitinated cargo and localizing to the endosomal membrane *via* interactions with phosphatidylinositol-3-phosphate (PI3P), as well as clathrin binding domains (Fig. 2A). The ESCRT-0 components are then able to recruit the ESCRT-I complex by interaction with the tumor susceptibility gene 101 subunit (Tsg101). The ESCRT-I assembly can then recruit ESCRT-II to the limiting endosomal membrane. ESCRT-II is able to recruit and activate the ESCRT-III machinery (Fig. 2B). ESCRT-II connects the ESCRT-I and ESCRT-III machinery by having one end of the complex, the vacuolar protein sorting-associated protein (Vps) 36 subunit, interact with ESCRT-I and ubiquitin, while the other end (two Vps25 subunits) bind the Vps20 subunit of ESCRT-III [10]. Activation of ESCRT-III results in the formation of Snf7 oligomers that cause budding of the vesicle into the endosome lumen (Fig. 2C); then ALG-2 interacting protein-X (ALIX) is recruited to stabilize ESCRT-III and deubiquitinate the proteins. After budding, the ATPase Vps4 is needed to provide energy to allow for disassembly and recycling of the ESCRT machinery [11–13]. There are other non-ubquitinated/ESCRT pathways including ceramide and lipid rafts. Inhibition of neutral sphingomyelinase (N-Smase2), which is responsible for ceramide production, blocks exosome production [14–16]. Ceramide is essential to create curvature of the endosomal membrane, thus facilitating ILV budding [14] (Fig. 2C).

1.2. Exosome fate: release vs. degradation

While there are multiple routes to MVB biogenesis and exosome release, it is unknown how and if they play a role in the eventual fate of MVBs, potentially regulating the exosome release *versus* exosome-lysosome fusion leading to degradation of the exosomal content. MVBs are trafficked to the plasma membrane with the help of the Rab GTPase family of proteins [17–19]; Rab5 and Rab7 are common markers of early and late endosomes respectively, while inhibition of Rab27 GTPases has been shown to block exosome secretion [18]. Silencing of Rab27a and Rab27b resulted in inhibition of docking with the cellular membrane and redistribution to the perinuclear space [18]. Rab27 overexpressing tumors show increased invasive properties in metastasis linked to output of exosomes to establish pre-metastatic niches [20–22]. Membrane fusion of the MVB is carried out by interactions of the vesicular soluble *N*-ethylmaleimide-sensitive factor-attachment protein receptors (SNAREs) with the intracellular target SNAREs allowing for release of exosomes [23,24].

1.3. Mechanisms of exosome uptake

Studies of exosome uptake indicate multiple different mechanisms of uptake, which may be due to both dissimilarities in recipient cells and in exosome composition [25]. A common mechanism of internalization is *via* endocytosis. Exosome uptake occurs rapidly and in an energy-requiring manner [26–28]. Inhibition of endocytic pathways by actin filament depolymerization, significantly reduces exosome uptake, but does not fully prevent it [29,30]. Both caveolin-mediated and clathrin-

Table 1
Shedding vesicle characteristics.

Particle	Size (nm)	Source	Common markers
Exosome	30–100	Internal budding of multivesicular bodies; released by fusion of MVB with cell membrane	Tetraspanins (CD63, CD81, CD9) TGS 101 Alix HSP 70
Shedding microvesicle	100–1000	Budding from cellular membrane	CD40 Selectin Intergrin
Apoptotic body	1000–5000	Blebbing from apoptotic cell membrane	Fragmented DNA Annexin V