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Review

Targeted therapeutic delivery using engineered exosomes and its applications in cardiovascular diseases

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ABSTRACT

Exosomes are 30–120 nm membrane bound vesicles secreted naturally by almost all cells and exist in all body fluids. Accumulating evidence has shown that exosomes contain proteins, lipids, DNA, mRNA, miRNA, and lncRNA that can be transferred from producer cells to recipient cells, facilitating cell–cell communication. As the natural carrier of these signal molecules, exosomes possess many other properties such as stability, biocompatibility, biological barrier permeability, low toxicity, and low immunogenicity, which make them an attractive vehicle for therapeutic delivery. How exosomes target recipient cells in vivo remains largely unknown, however, exosomes are selectively enriched in some transmembrane proteins that can be genetically engineered to display ligands/homing peptides on their surface, which confers exosome targeting capability to cells bearing cognate receptors. With the discovery of many peptides homing to diseased tissues or organs through phage display and in vivo biopanning technologies, there is ample opportunity to explore the potential use of exosome for targeted gene therapy. Here, we briefly review exosome biogenesis, mechanisms of exosome-mediated cell–cell communication, and exosome isolation and purification methods, and specifically focus on the emerging exosome targeting technologies.

Contents

Abbreviations: miRNA, microRNA; siRNA, small interference RNA; lncRNA, long non-coding RNA; ILVs, intraluminal vesicles; MVBs, multivesicular bodies; CD, cluster of differentiation; HSP, heat shock protein; SDCBP, syndecan binding protein; EBV, Epstein–Barr virus; RVG, rabies virus glycoprotein; iRGD, internalizing arginine–glycine–aspartic acid peptide; ORF, open reading frame; PCR, polymerase chain reaction; EGF, epidermal growth factor; PC, prostate cancer; LAMP-2b, lysosome-associated membrane protein-2B; PDGFR, platelet derived growth factor receptor; AA, aminoacid; RAG2, recombination activating gene-2; MFGE8, milk fat globule-EGF factor 8; OVA, ovalbumin; HER2, human epidermal growth factor receptor 2; CEA, carcinoembryonic antigen; PSA, prostate-specific antigen; PAP, prostatic acid phosphatase; ExoMAb, exosome monoclonal antibody; GAPDH, Glyceraldehyde 3-phosphate dehydrogenase; BACE1, beta-site amyloid precursor protein cleaving enzyme 1; UTR, untranslated region.

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1. Introduction

Exosomes are lipid bilayer enclosed nanovesicles secreted by almost all cells and exist in all body fluids (Keller et al., 2006; Madison et al., 2014; Vella et al., 2008; Ogawa et al., 2011; Choi, 2015). Originally thought as a mechanism cells use to remove unwanted cellular components (Pan & Johnstone, 1983), they are now recognized to be natural carriers of many signal molecules including lipids, proteins, DNA, mRNAs, miRNAs, siRNA, and lncRNAs that mediate cell–cell communication and thus play critical roles in physiology and in pathogenesis of cancer, autoimmune disease, diseases of infection, and cardiovascular diseases, among others (Denzer et al., 2000; Stahl & Barbieri, 2002; Ogorevc et al., 2013; Schorey & Bhatnagar, 2008). Exosomes are nanosized vesicles that are stable, biocompatible, low immunogenic, non-mutagenic, and biological barrier permeable (Boukouris & Mathivanan, 2015; Lakhal & Wood, 2011; Kooijmans et al., 2012; O'Loughlin et al., 2012), rationalizing their potential use as an ideal vehicle for therapeutic delivery. In exosome biogenesis, plasma membrane proteins and molecules existing in the cytoplasm are actively selected into exosome compartment through some not fully understood mechanisms. Most components enriched in exosome compartment are molecules that are common to all exosomes irrespective of the origin of their producer cells, while a small set of cellular components enriched are cell-type specific molecules reflecting the nature and the pathophysiological states of the specific producer cells (Keller et al., 2006; Schorey & Bhatnagar, 2008; Mathivanan et al., 2010; Vlassov et al., 2012). These enriched producer cell-specific molecules have been extensively exploited for vaccine development (Hartman et al., 2011; Rountree et al., 2011), disease diagnostic biomarkers (Hessels & Schalken, 2013; Keerthikumar et al., 2015; Tian et al., 2014; Alvarez-Erviti et al., 2011), and cancer immunotherapy (Fan, 2014; Delcayre et al., 2005; Fais et al., 2013; Cheng et al., 2014) and those enriched molecules common to all exosomes, such as transmembrane proteins, Lactadherin, lysosome-associated membrane protein-2B (LAMP-2b), and platelet derived growth factor receptor (PDGFR) have been exploited as targeting moieties to address ligands/homing peptides to the surface of exosomes (Alvarez-Erviti et al., 2011; Cooper et al., 2014) for the development of targeted gene delivery vehicles.

Exosome surface display has begun to emerge as novel theranostic tools in biomedicine. Molecules addressed to the surface of exosomes have been shown to confer targeting capability, and to have increased levels of expression, solubility, and antigen immunogenicity. For example, a G-protein-coupled receptor, when displayed on the surface of exosomes, showed increased solubility/functionality and had been used for drug screening (Estelles et al., 2007); ligand fragments/homing peptides addressed on the surface conferred exosome targeting capability, which had been used for targeted delivery of therapeutics including siRNAs, miRNAs, and chemotherapy drugs (Tian et al., 2014; Alvarez-Erviti et al., 2011; El-Andaloussi et al., 2012; Ohno et al., 2013); and tumor-specific antigens addressed on the surface of exosomes showed better antigen immunogenicity, which had been used to develop cancer vaccines and to produce monoclonal antibodies, a technology specifically called ExoMAb (Hartman et al., 2011; Delcayre et al., 2005). Here we briefly review exosome biogenesis, mechanisms of exosome-mediated cell–cell communication, exosome isolation techniques, exosome loading technologies, and routes of exosome delivery, and focus primarily on the technologies that address ligand/homing peptides on the surface of exosomes for targeted therapeutics delivery and touch on its applications in cardiovascular diseases.

1.1. Exosome biogenesis, mechanisms of trafficking, and exosome cargos

Exosomes are originated by two successive invaginations of plasma membrane (Schorey & Bhatnagar, 2008; Mathivanan et al., 2010; Vlassov et al., 2012): the inward budding of plasma membrane that forms endosomes, followed by the invagination of parts of endosome membrane toward the lumen of the endosomes, which selectively encapsulate molecules existed in the cytoplasm forming intraluminal vesicles (ILVs). The endosomes containing the newly-formed ILVs are now called multivesicular bodies (MVBs). When MVBs fuse with plasma membrane, the ILVs are released from producer cells as exosomes (Denzer et al., 2000; Sahoo & Losordo, 2014). Once released, exosomes can stay local or spread through body fluids to distal sites to affect functions of the recipient cells through one or the combination of the following mechanisms: (1) ligands or ligand-like transmembrane proteins on the surface of exosomes bind to their cognate receptors of either recipient cells or the producer cells (autocrine mechanism), and activate specific signaling pathways (Villarroya-Beltri et al., 2014; Munich et al., 2012; Raimondo et al., 2015) (2) exosomes fuse with the plasma membrane of recipient cells and directly release their cargos into the cytoplasm of the recipient cells (Mulcahy et al., 2014) (3) exosomes can be uptaken by recipient cells through macropinocytosis, endocytosis, or phagocytosis. These endocytosed exosomes can be released again or be degraded as endosomes mature into lysosomes (Mulcahy et al., 2014; Marcus & Leonard, 2013; Zhang et al., 2015) and (4) accumulating evidence now points to a new mechanism, exosomes burst and release their cargos, usually in low pH environment, before reaching the recipient cells, and the released cargos affect functions of the recipient cells through a paracrine mechanism (Taraboletti et al., 2006).

From the two successive invaginations theory of exosome biogenesis, one can easily infer that exosome membrane is the same as that of the plasma membrane of the producer cells. However, exosome membrane, other than having the same orientation as that of the producer cells, is selectively enriched in cholesterol, sphingolipids, glycerophospholipids, ceramide, and membrane proteins including endosome-associated proteins (Alix and Tsg101), tetraspanins (CD63/Lamp3, CD9, CD81, CD82), proteins that associated with lipid raft (flotillin and glycosylphosphatidylinositol-anchored protein), and others (heat shock protein-70, HSP70 and integrins) (Villarroya-Beltri et al., 2014; Klein-Scory et al., 2014; Raposo & Stoorvogel, 2013). The molecules encapsulated into ILVs are not a random but a selective regulated process, leading to the profile of cargo molecules inside the exosomes, albeit reflecting the nature and pathophysiological state of the producer cells, is not the same as that existed in the cytoplasm of the producer cells (Moreno-Gonzalo et al., 2014). The mechanisms of how molecules are selectively packaged into exosome compartment has begun to unfold, which is beyond the scope of this review and readers are encouraged to consult reviews by others (Villarroya-Beltri et al., 2014; Zhang et al., 2015; Stoorvogel, 2015).

1.2. Exosome isolation and purification

As natural circulating carriers of signal molecules that mirror the contents of the source cells, exosomes have great potential to develop into non-invasive diagnostics and therapeutic delivery vehicles. To qualify as a theranostic tool, standard protocols that allow for large-scale, high-purity, and high-quality isolation of exosomes have to be developed. Since the discovery of exosomes 30 years ago, several purification methods have been developed. The original and the most commonly used protocol is differential and high speed ultracentrifugation (Raposo et al., 1996), where live cells, dead cells, and cell debris were removed by differential centrifugations and the resulting supernatant was ultracentrifuged to pellet exosomes. The method itself is easy to perform, produce high yields, and can be easily scaled-up, but it is labor-intensive, requires ultracentrifuge, and the final exosome preparations contain many protein aggregates and other biomolecules co-precipitated with exosomes that may interfere with the downstream applications. Taking advantage of the specific density, exosomes can also be purified by density-gradient ultracentrifugation (Thery et al., 2006) that generally produce exosome preparation free of aggregates,