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Exosomes: Therapy delivery tools and biomarkers of diseases

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ABSTRACT

Virtually all cells in the organism secrete extracellular vesicles (EVs), a heterogeneous population of lipid bilayer membrane-enclosed vesicles that transport and deliver payloads of proteins and nucleic acids to recipient cells, thus playing central roles in cell-cell communications. Exosomes, nanosized EVs of endosomal origin, regulate many pathophysiological processes including immune responses and inflammation, tumour growth, and infection. Healthy subjects and patients with different diseases release exosomes with different RNA and protein contents into the circulation, which can be measured as biomarkers. The discovery of exosomes as natural carriers of functional small RNA and proteins has raised great interest in the drug delivery field, as it may be possible to harness these vesicles for therapeutic delivery of miRNA, siRNA, mRNA, lncRNA, peptides, and synthetic drugs. However, systemically delivered exosomes accumulate in liver, kidney, and spleen. Targeted exosomes can be obtained by displaying targeting molecules, such as peptides or antibody fragments recognizing target antigens, on the outer surface of exosomes. Display of glycosylphosphatidylinositol (GPI)-anchored nanobodies on EVs is a novel technique that enables EV display of a variety of proteins including antibodies, reporter proteins, and signaling molecules. However, naturally secreted exosomes show limited pharmaceutical acceptability. Engineered exosome mimetics that incorporate desirable components of natural exosomes into synthetic liposomes or nanoparticles, and are assembled using controllable procedures may be more acceptable pharmaceutically. In this communication, we review the current understanding of physiological and pathophysiological roles of exosomes, their potential applications as diagnostic markers, and current efforts to develop improved exosome-based drug delivery systems.

Contents

Abbreviations: AAV, adeno-associated virus; APC, antigen-presenting cell; CNS, central nervous system; CSF, cerebrospinal fluid; DC, dendritic cell; EBV, Epstein-Barr virus; EGFR, epidermal growth factor receptor; EEF, eukaryotic elongation factors; ESCRT, endosomal-sorting complex required for transport; EV, extracellular vesicle; GFP, green fluorescent protein; GM-CSF, granulocyte/monocyte colony-stimulating factor; GPI, glycosylphosphatidylinositol; GP1, glypican-1; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HSP, heat shock protein; HSPG, heparan sulfate proteoglycan; HSV, herpes simplex virus; ICAM, intercellular adhesion molecule; IL, interleukin; ILV, intraluminal vesicle; IFN-α, interferon-α; LAMP, lysosome-associated membrane protein; LFA-1, lymphocyte function-associated antigen-1; lncRNA, long noncoding RNA; MAGE, melanoma antigen gene; MHC, major histocompatibility complex; mRNA, messenger RNA; miRNA, microRNA; MRP-1, multidrug resistance-associated protein-1; MSC, mesenchymal stem cell; MVBs, multivesicular body; OVA, ovalbumin; PDGFR, platelet-derived growth factor receptor; PrPTSE, transmissible spongiform encephalopathy-associated prion protein; RVG, rabies viral glycoprotein; siRNA, small interfering RNA; tdTomato, tandem dimer Tomato; TGFβ, transforming growth factor-β; TIM-4, T-cell immunoglobulin- and mucin-domain-containing molecule-4; TLR, Toll-like receptor; TNF, tumour necrosis factor; TSG101, tumour susceptibility gene 101; VEGF, vascular endothelial growth factor; VPS4, vacuolar protein sorting-associated protein 4; VSV-G, vesicular stomatitis virus-G.

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1. Introduction

First described as small vesicles by which maturating sheep reticulocytes discard obsolete cellular components (Johnstone, Adam, Hammond, Orr, & Turbide, 1987; Pan & Johnstone, 1983; Trams, Lauter, Salem, & Heine, 1981), exosomes and other secreted extracellular vesicles (EVs) are now considered a prominent and universal form of cell–cell communication. Fundamentally all cells in the organism release EVs that are taken up by surrounding cells or circulate in the blood and eventually are taken up by cells at a distance. EVs transport biologically active molecules including proteins and nucleic acids that regulate gene expression and cellular function in target cells. As such, EVs mediate autocrine, paracrine, and endocrine effects that can be exploited therapeutically (Andaloussi, Mager, Breakefield, & Wood, 2013). For example, mesenchymal stem cells (MSCs) and other progenitor cells used in cell therapy studies mediate cytoprotective, angiogenic, and regenerative effects that are recapitulated by the EVs they release (Baglio, Pegtel, & Baldini, 2012; Barile et al., 2014). This observation raises the exciting prospect of "cell therapy without the cells". Efforts to harness EVs as carriers of signaling molecules for therapeutic applications have been focused on exosomes, nanosized EVs of endosomal origin. Another area of intense investigation is the use of EVs as biomarkers of disease. Healthy subjects and patients with different diseases secrete EVs with different contents into the circulation and bodily fluids, which can be measured for diagnostic purposes.

To contextualise exosomes as potential biotherapeutics and drug delivery vectors within the broader field of vesicle biology, current knowledge of their biogenesis, composition, and functional roles in health and disease is summarized in the initial part of this communication. Potential roles of exosomes as indicators of diseases and novel exosome-based drug delivery systems will then be addressed. More comprehensive information on the classification, composition, and functions of EVs can be found at http://www.isev.org (International Society for Extracellular Vesicles), http://www.asemv.org (American Society for Exosomes and Microvesicles), http://microvesicles.org (Vesiclepedia, a compendium for EVs with continuous community annotation) (Kalra et al., 2012), http://www.exocarta.org (ExoCarta, a web-based compendium of exosomal cargo) (Keerthikumar et al., 2016), and http://exrna.org (Extracellular RNA communication program).

2. Vesicle classes

The classification of EVs as a heterogeneous mixture of membrane particles has been inconsistent and somewhat confusing. Three major populations have been distinguished: (i) exosomes, initially defined as 50–100 nm lipid bilayer particles released from cells; the size range was then increased to include particles as small as 20 nm in diameter and those as large as 150 nm in diameter, although a size range of 30–100 nm was used in most studies; (ii) microvesicles, also referred to as shedding vesicles (Shedden, Xie, Chandaroy, Chang, & Rosania, 2003) or microparticles (Shantsila, Kamphuisen, & Lip, 2010), which tend to be larger than exosomes (size range: 50–1000 nm); (iii) apoptotic bodies (size range: 50–5000 nm), which result from the fractionation of the cellular content of cells that die by apoptosis (Cline & Radic, 2004; Hristov, Erl, Linder, & Weber, 2004). As apparent, these populations of EVs overlap in size. As a result of improved understanding of their biogenesis, the origin of EVs has become an important qualifier for their identity. Accordingly, exosomes are of EVs endosomal origin (Simons & Raposo, 2009), whereas microvesicles typically form by membrane shedding, especially from injured or transformed cells (Muralidharan-Chari, Clancy, Sedgwick, & D'Souza-Schorey, 2010).

Based on comparative proteomics analyses, heterogeneous EV population can be subdivided into large, medium-sized, and small vesicles pelleting at low (2000 $\times g$), intermediate (10,000 $\times g$), and high (100,000 $\times g$) sedimentation speed, respectively, the latter being the ultracentrifugation pellet classically considered as containing exosomes. Both exosomal and nonexosomal subpopulations were present within small vesicles (mean size < 200 nm), which were further subdivided into four categories: (i) *bona fide* exosomes coenriched in exosome (CD63, CD81, CD9) and endosome markers such as syntenin-1 and tumour susceptibility gene 101 (TSG101); (ii) small EVs devoid of CD63/CD81 but enriched in CD9; (iii) small EVs devoid of CD63/CD9/CD81; (iv) small EVs enriched in extracellular matrix proteins or serum-derived factors (Kowal et al., 2016). For now, it is important to recognize that the nomenclature tends to be used rather loosely, and that it may be difficult in practice to distinguish exosomes from other EVs.

3. Biogenesis of exosomes

Endosomes arise from invaginations of the plasma membrane and fuse with molecular payloads sorted in the endoplasmic reticulum and processed in the Golgi complex, forming multivesicular endosomes (also referred to as multivesicular bodies; MVBs). When MVBs mature and eventually merge with the plasma membrane, their content is released into the extracellular space as exosomes (Fig. 1). Rab GTPase proteins regulate fusion of MVBs with the cell membrane (Pfeffer, 2010) and the spatio-temporal traffic of vesicles (Stenmark, 2009). Endosomal-sorting complex required for transport (ESCRT) is the central molecular machinery of exosome formation at endosomes (Février & Raposo, 2004; Hurley, 2010; Raiborg & Stenmark, 2009; Stoorvogel, 2015). The ESCRT system comprises four multi-protein complexes (ESCRT-0, -I, -II, -III), vacuolar protein sorting-associated protein 4 (VPS4)-Vta1, and the Alix homodimer. ESCRT-0 participates in cargo clustering and ubiquitination of endocytosed receptors (Katzmann, Odorizzi, & Emr, 2002; Katzmann, Stefan, Babst, & Emr, 2003). On endosomes, ubiquitinated membrane proteins enter the MVB pathway whereas nonubiquitinated proteins are recycled back to the plasma membrane or to the Golgi complex. TSG101, a component