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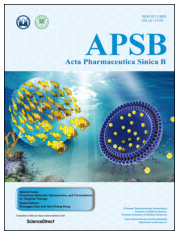

REVIEW

Exosomes as therapeutic drug carriers and delivery vehicles across biological membranes: current perspectives and future challenges

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KEY WORDS

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Abstract Exosomes are small intracellular membrane-based vesicles with different compositions that are involved in several biological and pathological processes. The exploitation of exosomes as drug delivery vehicles offers important advantages compared to other nanoparticulate drug delivery systems such as liposomes and polymeric nanoparticles; exosomes are non-immunogenic in nature due to similar composition as body's own cells. In this article, the origin and structure of exosomes as well as their biological functions are outlined. We will then focus on specific applications of exosomes as drug delivery systems in pharmaceutical drug development. An overview of the advantages and challenges faced when using exosomes as a pharmaceutical drug delivery vehicles will also be discussed.

Abbreviations: ALIX, ALG-2 interacting protein X; ATPase, adenosine triphosphatase; BBB, blood–brain barrier; CCK-8, cell counting kit-8; CD, cluster of differentiation; DIL, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate; DNA, deoxyribonucleic acid; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; EpCAM, epithelial cell adhesion molecule; ESCRT, endosomal sorting complexes required for transport; EV, extracellular vesicle; HEK293, human embryonic kidney cell line 293; HeLa, Henrietta Lacks cells; HIV, human immunodeficiency virus; HMGA2, high-mobility group AT-hook protein; Hsp, heat shock proteins; IL-6, interleukin-6; ILVs, intraluminal vesicles; kRAS, Kirsten rat sarcoma; LPS, lipopolysaccharides; MAPK-1, mitogen-activated protein kinase 1; MHC, major histocompatibility complex; miRNA, micro RNA; MPS, mononuclear phagocyte system; mRNA, messenger RNA; MVB, multi-vesicular body biogenesis; PBMC, peripheral blood mononuclear cells; PD, Parkinson's disease; PEG, polyethylene glycol; RNA, ribonucleic acid; ROS, reactive oxygen species; RPE1, retinal pigment epithelial cells 1; siRNA, small interference RNA; TNF-α, tumor necrosis factor α; TSG101, tumor susceptibility gene 101; VPS4, vacuolar protein sorting-associated protein 4

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1. Introduction

Intercellular communication is absolutely essential to cell development and the maintenance of homeostasis in multicellular organisms. These communications between cells can be localized or distant. Local communication involves direct contact between cells facilitated through communication systems, like a gap junction that connects the cytoplasm of cells adjacent to one another, allowing signaling substances to pass between the cells. On the other hand, distant intercellular communication is facilitated by molecules like hormones that send signals through circulatory system to other parts of the body. Another case of distant intercellular communication also occurs in extracellular vesicle (EV), which is a membrane-based structure. These EVs serve as vehicles to carry different types of cellular cargo—such as lipids, proteins, receptors and effector molecules—to the recipient cells[1].

There are three types of EVs that are differentiated based on their intracellular origins: apoptotic bodies, microvesicles and exosomes[2]. Apoptotic bodies have a size ranging from 50 to 5000 nm and contain cellular contents such as deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and histone proteins. During apoptosis, apoptotic bodies present these contents to marcrophages, which results in cell engulfation[3,4]. Other types of EVs known as microvesicles are also referred to as ectosomes, shedding vesicles, microparticles, plasma membrane-derived vesicles, and exovesicles. Microvesicles are formed through the outward budding and fission from plasma membranes, with a size ranging from 50 nm to 1000 nm. Once microvesicles are formed, they carry specific proteins and lipids and then deliver their cargoes to designated recipient cell[2]. The final category of EVs is exosomes, which differ from microvesicles mainly in terms of their intracellular origin and size (Fig. 1).

Over the past decades, there has been extensive research carried out on exosomes. The word "exosome" was first used in 1970 by Rose Johnstone and her colleagues[5]. While working with maturing reticulocytes, they observed the formation of "an intracellular sac filled with small membrane-enclosed structure of nearly uniform size". These formed intracellular vesicles and released contents outside the cell, as opposed to inside of cell (endocytosis), where external molecules are internalized into the membrane-bound structure. Hence, these intracellular-formed vesicles were named "exosome"[5].

Exosomes are originated from endosomes with a smaller size, ranging from 40 to 100 nm[6]. They are secreted by all cell types and can be found in most body fluids, including blood, saliva, and urine. An exosome is a "nanosphere" with a bilayerd membrane, containing various types of lipids and proteins derived from the parent cell. Some of these proteins include transport proteins, heat shock proteins, proteins associated with multi-vesicular body biogenesis (MVB), and tetraspanin. In addition to proteins, exosomes are comprised of different types of lipids, such as cholesterol, sphingolipids, phosphoglycerides, ceramides, and saturated fatty acid chains[7]. The composition of exosomes is critical since they serve as a biomarker and provide an indication of its function in biological processes.

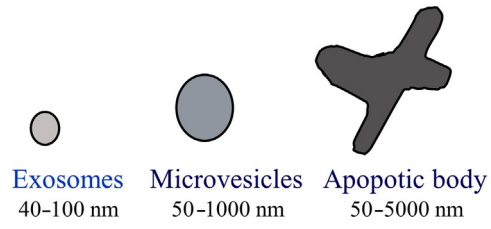

Figure 1 Types of microvesicles. Exosome with sizes ranging 40–100 nm (left), microvesicles with sizes ranging 50–1000 nm (middle), apoptotic body size ranging 50–5000 nm (right).

1.1. *Formation of exosomes*

In general, the formation of exosomes consists of three different stages: (1) the formation of endocytic vesicles from plasma membrane, (2) the inward budding of the endosomal vesicle membrane resulting in MVBs that consist of intraluminal vesicles (ILVs), and (3) the fusion of these MVBs with the plasma membrane, which releases the vesicular contents, known as exosomes[7] (Fig. 2). In the first stage, endocytic vesicles are formed from the plasma membrane, creating an early endosome, which is then matured into late endosomes. The limiting membrane of these late endosomes undergoes inward budding, in turn forming vesicles inside the lumen. The accumulation of these ILVs inside the late endosomes is termed as MVBs. There are two known pathways to the formation of MVBs. One pathway involves endosomal sorting complexes required for transport (ESCRT) and another pathway is ESCRT independent. These MVBs can either fuse with lysosome for degradation or fused with the plasma membrane of the cell, releasing ILVs into extracellular space and these released ILVs are exosomes[8].

The ESCRT consists of four soluble multi-protein complexes named ESCRT-0, ESCRT-I, ESCRT-II, and ESCRT-III. The complex is recruited to sort selected proteins into ILVs. ESCRT-0 is responsible for cargo clustering, which is required for the ubiquitination of endocytosed receptors[9]. These cargoes constitute proteins that will be incorporated into ILVs and, later, become part of the released exosomes. Tumor susceptibility gene 101 (TSG101), a component of ESCRT-I, forms a complex with the ubiquinated cargo protein and helps in the activation of ESCRT-II complex, inducing its bud formation. This complex then involves

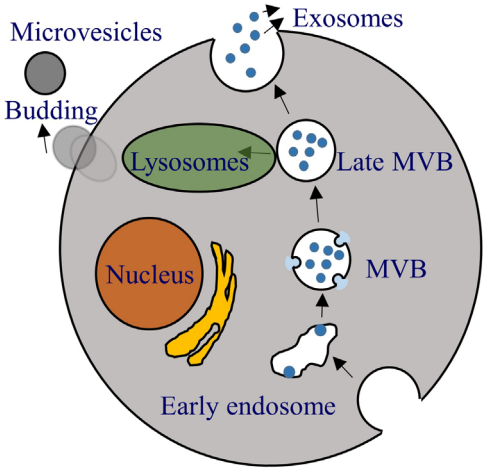

Figure 2 Formation of exosome and microvesicle. Exosome is derived from endosome formed from plasma membrane. As early endosome becomes late endosomes, inward budding occurs and forms multivesicular bodies (MVB) containing numerous intraluminal vesicles (ILV). MVB can either get degraded by lysosomes or fuse with the membrane to release ILV called exosomes. Micorvesicles, on the other hand, originate from the budding of the plasma membrane.