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Review article

Recent advances on extracellular vesicles in therapeutic delivery: Challenges, solutions, and opportunities

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ABSTRACT

Extracellular vesicles (EVs) are intrinsic mediators of intercellular communication in our body, allowing functional transfer of biomolecules (lipids, proteins, and nucleic acid) between diverse locations. Such an instrumental role evokes a surge of interest within the drug delivery community in tailoring EVs for therapeutic delivery. These vesicles represent a novel generation of drug delivery systems, providing high delivery efficiency, intrinsic targeting properties, and low immunogenicity. In the recent years, considerable research efforts have been directed toward developing safe and efficient EV-based delivery vehicles. Although EVs are shown to harbor great promise in therapeutic delivery, substantial improvements in exploring standardized isolation techniques with high efficiency and robust yield, scalable production, standard procedures for EV storage, efficient loading methods without damaging EV integrity, understanding their *in vivo* trafficking, and developing novel EV-based nanocarriers are still required before their clinical transformation. In this review, we seek to summarize the recent advance on harnessing EVs for drug delivery with focus on state-of-the-art solutions for overcoming major challenges.

Contents

Abbreviations: EVs, extracellular vesicles; MVBs, multivesicular bodies; MPS, mononuclear phagocyte system; ILVs, intraluminal vesicles; ESCRT, endosomal sorting complex required for transport; GTP, guanosine triphosphatase; SNARE, soluble N-ethylmaleimide-sensitive factor attachment protein receptors; MHC, major histocompatibility complexes; HIV, human immunodeficiency virus; ISEV, International Society for Extracellular Vesicles; UC, ultracentrifugation; UC-G, density gradient centrifugation; SEC, size-exclusion chromatography; UF, ultrafiltration; PEG, polyethylene glycol; IC, Immunoaffinity capture; EpCAM, epithelial cell adhesion molecule; Alix, ALG-2-interacting protein X; TSG101, tumor susceptibility gene 101; nano-DLD, nanoscale deterministic lateral displacement; MSCs, mesenchymal stem cells; HFBRs, hollow-fiber bioreactors; CD-UPRT, cytosine deaminase fused to uracil phosphoribosyltransferase; 5-FC, 5-fluorocytosine; EXPLORs, exosomes for protein loading via optically reversible proteinprotein interactions; iRGD, integrin-specific peptide; RVG, rabies virus glycoprotein; Lamp2b, lysosome-associated membrane glycoprotein 2b; TGF-β1, transforming growth factor β1; HEK 293, human embryonic kidney cell 293; BBB, blood brain barrier; CagA, cytotoxin-associated gene A; AAVs, adeno-associated viruses; EPNs, enveloped protein nanocages; PD, Parkinson's disease.

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1. Introduction

Intercellular communications in multi-cellular organisms are generally accomplished by direct cell-cell contact and releasing soluble mediators, such as hormones and cytokines. However, a third way for intercellular communication which has been less well understood is vesicle exchange. EVs are membrane-derived vesicles secreted by (almost) all cell types of both prokaryotic and eukaryotic organisms and can be categorized into three subtypes (i.e. exosomes, microvesicles, and apoptotic bodies), according to their size and pathway of origin [1]. Exosomes (40–100 nm), originating from intraluminal budding of multivesicular bodies (MVBs), are the most comprehensively studied EVs [2]. Slightly larger and more heterogeneous than exosomes, microvesicles (100 nm–1 μm) are formed by directly budding from the plasma membrane. Although exosomes and microvesicles are of different biogenesis, they share similar physicochemical features [3]. Apoptotic bodies with a micro-sized diameter of 1–5 μm are released from cells undergoing apoptosis. By wrapping fragments of the nucleus and cytoplasm in plasma membrane, apoptotic bodies prevent the spilling out of cytotoxic contents into extracellular environment [4]. Currently, there are few subtype-specific markers to discriminate these vesicles and it remains difficult for state-of-the-art isolation protocols to distinguish different types of vesicles [5]. However, the relatively large size of apoptotic bodies rules out their potential as therapeutic carriers [6]. Therefore, EVs mainly refer to exosomes and microvesicles in this review.

The secretion of EVs is initially regarded as a waste disposal mechanism. In 1967, a first report on EVs described that 'platelet dust' bearing coagulant activity was shed from platelets. The dust was further identified to be membranous vesicles and named microparticles [7]. Later on, [8] reticulocytes were observed to release vesicles bearing transferring receptors during their maturation process and the term "exosomes" was introduced to describe these secreted vesicles. In 1996, EVs from B lymphocytes were demonstrated to present antigens, and thus trigger T cell responses [9]. These findings inspired an increasing number of researchers to explore the diagnostic and therapeutic applications of EVs. Recently, the fact that EVs contain several molecules that are specific to the type and status of their donor cells, leads to the idea of employing EVs as noninvasive biomarkers in diagnostics [10]. Furthermore, the key function of EVs to transfer shuttled contents between cells has fueled the idea of tailoring EVs for drug delivery [11].

The advent of nanotechnology heralds a new chapter in drug delivery and gene therapy. Viral vectors have been applied to deliver genes with high efficiency. However, the potential for insertional oncogenesis, high production cost, and inducing immunogenicity limits their clinical use. Non-viral nanocarriers, such as liposomes and polymeric nanoparticles, are generally less mutagenic and immunogenic. Nevertheless, issues with low transfection efficiency, cytotoxicity, and poor biocompatibility are still major challenges when using non-viral nanocarriers [12]. In contrast, EVs provide a diverse range of unique advantages for serving as drug delivery vehicles (Table 1). EVs are nearly non-immunogenic when deriving from patient's own cells. Therefore, EV-based nanocarriers may act as "invisibility cloak" for loaded drugs, thus reducing clearance by mononuclear phagocyte system (MPS) [13]. In addition, EVs possess intrinsic targeting properties that can guide therapeutic cargos across natural membranous barriers [14]. Importantly, the specific lipid and protein compositions of EVs allow them to directly fuse with cell membranes and efficiently deliver their cargoes into the cytosol [15]. Therefore, this mode of internalization largely bypasses the endocytic pathway. Of note, the safety of EVs has been extensively tested and EVs were shown to be relatively safe in numerous clinical trials [16,17].

Although EVs hold great potentials in therapeutic delivery, some obstacles are ahead before reaching maximum potential in clinic (Table 1). In this review, we first give an overview of the fundamental biology of EVs, and then thoroughly examine state-of-