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REVIEW ARTICLE

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Exosomes: Nanoparticulate tools for RNA interference and drug delivery

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Exosomes are naturally occurring extracellular vesicles released by most mammalian cells in all body fluids. Exosomes are known as key mediators in cell-cell communication and facilitate the transfer of genetic and biochemical information between distant cells. Structurally, exosomes are composed of lipids, proteins, and also several types of RNAs which enable these vesicles to serve as important disease biomarkers. Moreover, exosomes have emerged as novel drug and gene delivery tools owing to their multiple advantages over conventional delivery systems. Recently, increasing attention has been focused on exosomes for the delivery of drugs, including therapeutic recombinant proteins, to various target tissues. Exosomes are also promising vehicles for the delivery of microRNAs and small interfering RNAs, which is usually hampered by rapid degradation of these RNAs, as well as inefficient tissue specificity of currently available delivery strategies. This review highlights the most recent accomplishments and trends in the use of exosomes for the delivery of drugs and therapeutic RNA molecules.

KEYWORDS
drug delivery, exosome, miRNA, siRNA, vesicle

1 | INTRODUCTION

Exosomes (endogenous nanocarriers that are 30–120 nm in diameter) are secreted by a variety of cell types, such as epithelial, dendritic, and tumor cells (Mu, Rana, & Zöller, 2013). Such nanocarriers are also present in body fluids such as blood, urine, amniotic effusions, malignant ascites, bronchoalveolar lavage fluid, synovial fluid, and breast milk. Additionally, they have been found in the supernatants of different cell types grown in culture (Mu et al., 2013; Simpson, Jensen, & Lim, 2008; Wang et al., 2014). Such nanocarriers were first described 25 years ago in mature sheep reticulocytes involved in the externalization of the transferrin receptor (Simpson et al., 2008). These vesicles can deliver biological information between cells through various surface adhesion proteins and ligands including tetraspanins, integrins, and CD11b and CD18 receptors (Batrakova and Kim, 2015). Exosomes are also involved in the presentation of antigens to T cells, transfer of surface receptors from one cell to another, signal transduction, and the development of tolerance (Théry et al., 2002; Vlassov, Magdaleno, Setterquist, & Conrad, 2012). They are formed from multivesicular bodies (MVBs) created by inward budding of late endosomes in the form of isolated intraluminal vesicles (ILV) and subsequently released to the extracellular milieu upon fusion with plasma membranes. The lipid-driven pathway, which uses the endosomal sorting complexes required for transport (ESCRT) system together with ESCRT-independent pathways, is critically involved in ILV formation (Wang et al., 2014). Sphingolipid ceramide and the phospholipid lysobisphosphatidic acid (LBPA) are considered important lipids in the process of ILV formation, which facilitates the inward

transformation of the membrane. Evidently, lipids contribute to the creation of ILVs out of MVBs, but, on the other hand, ESCRTs are involved in cargo sorting (Batrakova & Kim, 2015). Moreover, plasma membrane proteins and cytoplasmic molecules are incorporated into the exosomes during its biogenesis, but this mechanism is not fully understood. Exosomes share some structural elements irrespective of the origin of the cell that produced them, while some molecules are specific to a cell-derived exosome and reflect the physiological state of the cell that produced them (Schorey & Bhatnagar, 2008; Vlassov et al., 2012). These nanocarriers also confer an endocytic pathway for protein export through scission from the limiting endosome, which result in cell type specific proteins enclosed in the membrane of the parent cell, with the unique property of homing selectivity and the same topological orientation (Green, Langer, & Anderson, 2008; Simpson et al., 2008). These cell type-specific proteins determine an exosome's functionality (Simpson et al., 2008). Hence, transmembrane proteins, such as lactadherin, lysosome associated membrane protein-2B (LAMP-2b), platelet derived growth factor receptor (PDGFR), heat shock protein, and annexins are common to all exosomes and can be utilized for targeted gene delivery associated with homing peptides on the surface of exosomes (Alvarez-Erviti et al., 2011; Vlassov et al., 2012; Xitong & Xiaorong, 2016). In addition, exosomes have specific proteins that distinguish them from microvesicles, such as alix, TSG101, and tetraspanins (CD9, CD63) (Simpson et al., 2008). Initially, exosomes were thought to be a cellular mechanism to remove unwanted components. However, exosomes are now considered as a natural carrier of many signaling molecules, and thus play an important role in the pathogenesis of many diseases involved with the immune system, sepsis, cardiovascular, and cancer (Ogorevc, Kralj-Iglic, & Veranic, 2013). Furthermore, exosomes and their modified variants can be used as vectors for cancer therapy by the delivery of drugs, microRNAs (miRNAs), small interfering RNAs (siRNAs), mitochondrial DNA, genomic DNA, and recombinant proteins (Banizs et al., 2014). Therefore, tumor-associated exosomes may represent an important biological defense and regulatory mechanism during cancer development and progression. Moreover, exosome-mediated delivery of cell-derived cargos might provide tissue-specific biomarkers which would allow for the diagnosis of cancer growth at early stages, heart disease, pregnancy, and infections (Théry et al., 2002). In this review, we have chosen to focus on different delivery approaches of noncoding RNAs and therapeutic drugs using exosomes.

2 | siRNA AND miRNA DELIVERY WITH EXOSOMES

RNAi-based therapy, which is performed with both small interfering RNAs (siRNAs) and microRNAs (miRNAs), has been demonstrated to be one of the most important strategies for target-specific gene silencing (Kooijmans, Vader, van Dommelen, van Solinge, & Schiffelers, 2012). However, in spite of major advances in the field of RNAi, only a few clinical trials have been performed. Poor bioavailability, rapid hydrolysis, and the inability to cross biological barriers, such as blood-brain barrier, are major concerns for the successful clinical application of siRNAs (Schorey & Bhatnagar, 2008). Investigations have revealed that naked siRNA has a natural affinity for gene silencing in the spleen, kidney, and liver. Conversely, exosome-encapsulated siRNAs have no affinity to the aforementioned tissues, but undergo a more specific uptake (Ogorevc et al., 2013). It requires two critical steps to efficiently deliver miRNAs or siRNAs to a specific tissue while maintaining their expression capacity; targeting to the desired tissue and subsequent delivery across the cell membrane (Alvarez-Erviti et al., 2011). Many siRNA and miRNA delivery methods have been developed based on viral and non-viral vectors used for gene delivery. Although, viral delivery generally leads to long-term gene silencing in the target tissue, the safety of viral vectors is still a matter of concern. Viral delivery has inherent drawbacks, for example, it can result in the activation of complement or coagulant factors in the blood circulation. Moreover, viruses can be recognized by preexisting antibodies in the blood stream. There are also safety issues regarding dysregulation of gene expression in the desired tissue, which might lead to malignant transformation and several other complications, thus limiting the application of viral vectors in clinical practice (Green et al., 2008; Hornung et al., 2008). Non-viral delivery systems involving synthetic carriers, such as polyethyleneimmine (PEI) nanoparticles or liposomes, are promising alternatives because of their ability to encapsulate siRNAs, protect them from degradation in the bloodstream, and facilitate cellular uptake. However, although non-viral delivery systems have surpassed safety concerns when compared to viral vectors, there are potential problems related to inadequate transfection efficiency, induction of a pro-inflammatory response, and apoptosis in vivo (Green et al., 2008; Hornung et al., 2008; Kooijmans et al., 2012). Exosomes have some features that are common to liposomes including having a phospholipid bilayer and being composed of biocompatible substances (Dang Xitong, 2015). It has been established that exosomes have benefits of a natural vehicle for transferring siRNAs with high target specificity and lack of immunologic reactions. This nanocarrier also has the properties of efficient uptake in host cells due to their unique composition of endogenously synthetized lipid, protein, and RNA, which is not found in other delivery systems (Kooijmans et al., 2012). According to some reports, exosome-mediated delivery systems are well tolerated both in vitro and in vivo, as demonstrated by the MTT cytotoxicity test and immunological assessments (Ogorevc et al., 2013). In comparison with other existing gene therapy vehicles, repeated administration of exosomes did not activate the host immune response (Hornung et al., 2008). Hence, it has been suggested that exosomes have benefits of both synthetic nanocarriers and cell-mediated drug delivery systems, but lack most of their limitations.

Many studies have demonstrated the existence of RNA (such as mRNA and miRNA) in exosomes that were derived from different cell types, transferred miRNAs between cells, and consequently suppressed target genes (Adlakha & Saini, 2014; Kooijmans et al., 2012). Thus, exosomes have the advantage of being a nano-sized, cell-based delivery system that is considered an effective vehicle for the targeted delivery of both endogenous and exogenous payloads (Green et al., 2008). miRNAs are small noncoding RNAs that control specific target mRNAs and inhibit gene expression at either the post-transcriptional or

transcriptional level (Banizs et al., 2014). In contrast to siRNAs that are well-known for sequence specificity and used to regulate the expression of one specific gene, miRNAs regulate functionality of several genes related to a signaling pathway or even multiple cross-talking signaling pathways. These can increase their potential for off-target effects (Adlakha & Saini, 2014; Kooijmans et al., 2012). Similar to the limitations related to the delivery of siRNA, miRNA properties require a suitable delivery system. Endogenous miRNAs can be encapsulated into exosomes to protect them from endonuclease degradation. Although synthetic miRNAs have a short half-life in the blood stream, various chemical modifications can be utilized to improve their loading into exosomes (Dang Xitong, 2015; Kooijmans et al., 2012; Zhang, Wang, & Gemeinhart, 2013).

GW182 and argonaute2 (AGO2), which are considered as two main components of RNA-induced silencing complex (RISC), suggest that exosomes are the site for miRNA accumulation and, possibly, gene silencing (Montecalvo et al., 2012). Most importantly, the association of GW182 and AGO2 with MVBs may be involved with the sorting of miRNAs into exosomes (Gibbings, Ciaudo, Erhardt, & Voinnet, 2009; Zhang et al., 2013). Naturally occurring RNA carriers secreted from many tumor cells contain miRNAs, which facilitate cell-cell communication during carcinogenesis by environmental chemical induction. This process leads to proliferation in neighboring normal cells. Exosomes loaded with an miRNA inhibitor are considered an effective carrier system compared with conventional transfection methods used to treat disease (Wang et al., 2014). Thus, it has been suggested that exosomes can be used as a natural source of mRNA and miRNAs carriers (Banizs et al., 2014). Consequently, exogenous siRNAs and other types of RNAi, such as miRNAs mimics, can also be loaded into exosomes and transferred to recipient cells (Dang Xitong, 2015; El-Andaloussi et al., 2012). For therapeutic applications, such exosomes can be used both in either an unmodified, or engineered, form (Mu et al., 2013). Unmodified exosomes, which have been derived from various cell types, exhibit desirable therapeutic properties for various applications (Zitvogel et al., 1998). Although exosomes may be generated from many cell types (e.g., bone marrow, blood, monocytes, or macrophages), a few have been chosen for the delivery of drugs or short nucleic acids. Immature dendritic cells provide an untapped source of exosomes devoid of any T-cell activators and are a suitable source for vaccination purposes. Furthermore, exosomes derived from endothelial cells provide an appropriate source of exosomes to deliver siRNAs to endothelial cells without any unnecessary components from nonendothelial origin. Deriving exosomes from different types of cells may confer different compositions and functions (Banizs et al., 2014).

Exosomes may also be engineered to be incorporated with therapeutic cargo such as proteins, therapeutic RNAs, and conventional low-molecular-weight organic drug molecules in order to confer desirable therapeutic activities (Akao et al., 2011; Mu et al., 2013; Ogorevc et al., 2013).

Different strategies have been employed to describe exosome-based delivery systems. From the point of RNA therapy, exosomes can be loaded with therapeutic miRNAs and siRNAs using different methods. Electroporation represents just one broadly applicable method to introduce exogenous RNAs and other therapeutic molecules onto the surface of purified exosomes. Alvarez-Ervitiz and others have successfully utilized electroporation to introduce siRNA into dendritic cell (DC)-derived exosomes, which had been modified to express rabies virus glycoprotein (RVG)-derived peptide fused with the integral exosomal membrane protein, Lamp2b, to trigger neuronal cells to knock down expression of the desired gene in vivo (Alvarez-Erviti et al., 2011; Ogorevc et al., 2013). RVG exosomes could be used for long-term silencing of genes related to neurodegenerative disease. However, electroporation may not be effective for some configurations of RNAs such as miRNA and shRNA, which contain numerous chemical modifications (Dang Xitong, 2015). Therefore, the quality of exosomes prepared by electroporation depends on the conditions used, for example, buffers for resuspending the exosomes (van der Meel et al., 2014). The electroporation protocol used for loading siRNA into exosomes has revealed conflicting results and has been reported to be hampered by the negative charge of siRNA. Thus, siRNA complexes within a cationic liposome, followed by fusion with the exosome, may overcome many of the limitations associated with expulsion of the siRNA from the extracellular vesicle (Wang et al., 2014). Moreover, a slightly elevated temperature (37°C) may improve the loading of siRNAs into the exosome (van der Meel et al., 2014).

Another way for incorporating RNAs into exosomes is through overexpression of the cargo RNAs in the exosome-producing cells. These RNAs (miRNAs, modified miRNAs, and shRNA) were completely functional when incubated with recipient cells and resulted in target gene knockdown (Marcus & Leonard, 2013; Rechavi et al., 2009). This strategy may be combined with the expression of a tissue-targeted protein as a fusion protein with the surface of an exosomal protein. For example, cells can be co-transfected with two vectors, one which encodes the precursor miRNA, and the other which expresses fusion-targeted protein in order to display the targeting peptide on the surface of the exosome during purification, and then miRNAs are packed inside (Alvarez-Erviti et al., 2011).

Cell-penetrating peptides (CPP-exosome) is a good example with which to improve the overall uptake efficiency of exosomes. CPP exosomes, in which a short cationic peptide is fused with Lamp2b and displayed on the surface of the exosome, may be employed as an appropriate cargo that enters cells through the shield of negative charges of the exosome (El-Andaloussi, Holm, & Langel, 2005). Another strategy to introduce miRNAs into exosomes is transient transfection using commercial transfection reagents (Dang Xitong, 2015; Shtam et al., 2013). Exosome display technology is another method to introduce exogenous siRNAs successfully into different kinds of human exosomes (Wahlgren et al., 2012). To use exosomes as a potential therapeutic system, several issues need to be addressed. Studies have shown that exosomes can be easily cleared by the reticuluendothelial system (RES), since many of them accumulate in the liver 24 hr after injection into a mouse tail vein (Ohno et al., 2013). Therefore, several strategies have been developed to target exosomes to specific cellular receptors. One approach to precise delivery of exosome-mediated cargo to a specific cell type is to harness a

virus-derived protein or peptide to labeled exosomes. For instance, RVG-exosomes, which display a central nervous system-specific rabies viral glycoprotein, have the ability to deliver siRNA to neural cells by binding to the acetylcholine receptor. On the other hand, untagged exosomes deliver siRNAs to unintended tissues. Virus-modified exosomes represent a new approach to specific targeted delivery. Because there are many similarities between viruses and exosomes, exosomes may be modified by incorporating viral proteins, which may be exploited for specific targeting of the exosomes (Sun et al., 2010). Viral-infected cells that incorporate viral factors like virus-encoded RNA into exosomes could be efficiently delivered into non-infected target cells. Studies have revealed an enhancement of exosomes released from the infection of cells with Rotavirus than non-infected with higher T-cell inhibition, while no viral product was detectable (Barreto et al., 2010). These modified exosomes carrying viral proteins were incorporated into endogenously produced exosomes so as to increase the uptake and delivery of exosomes by the desired target (Alvarez-Erviti et al., 2011; Koppers-Lalic, Hogenboom, Middeldorp, & Pegtel, 2013). For instance, EBV-infected B cells encoded BART miRNAs were transferred through exosomes to multiple uninfected recipient cells including monocyte-derived DC. These EBV-miRNAs accumulated in noninfected recipient cells via exosomes and caused suppression of EBV target genes (Pegtel et al., 2010). Likewise, plasma exosomes, which were derived from the peripheral blood of healthy donors, were able to deliver siRNAs effectively into the target cells (human blood mononuclear cells) (Wahlgren et al., 2012). Exosome-mimetics represent a viable alternative method to deliver miRNA and siRNA directly into the cytoplasm of target cells. The aim of this biotechnological approach has been to synthetize exosomes with a less complex structure and harness only crucial components of natural exosomes required for specific and efficient delivery of the exosomes to the target tissue. Since liposomes have a spherical lipid bilayer structure in common with exosomes, it suggests a logical base for the creation of functional exosome-mimetics.

Recently, cell-derived exosome mimetics have been developed by utilizing cellular membranes of monocytes and macrophages, which were serially extruded through filters with diminishing pore sizes of 10, 5, and 1 μm. These manufactured lipid bilayered membrane vesicles share similar properties to those of exosomes while maintaining the natural targeting capacity and a topology of plasma membrane proteins (Jang & Gho, 2014). Furthermore, exosome-mimetics represent an excellent delivery system, and avoids nonimmunogenic and nontoxic delivery of therapeutic cargos (siRNA and miRNAs) to desired cells (Kooijmans et al., 2012; Puri et al., 2009). Additionally, these exosome-mimetics are a promising nanotechnology to overcome the hurdle of exosome purification and low production yields (Jang & Gho, 2014). In general, the overall efficiency of this exosome-mediated delivery strategy can be achieved through targeting to specific recipient cells and utilizing intracellular mechanisms such as intracellular trafficking and specific modes of exosomal uptake in order to confer the best delivery of the cargo molecules.

3 | APPLICATION OF EXOSOMES IN DRUG DELIVERY

The small size of exosomes is an advantage for their use as drug delivery systems, because this enables them to escape rapid clearance by the mononuclear phagocyte system (Xitong & Xiaorong, 2016). Moreover, exosomal drug delivery systems can provide unique benefits including stability in the blood due to bypassing the complement system, efficient delivery of drugs into the cytosol of target cells, and possibly fewer off-target effects due to the inherent biocompatibility of exosomes (Kooijmans et al., 2012).

Studies have shown that 22–25% of healthy donors have PEG antibodies in their blood due to the exposure to PEG used in cosmetics and foods. Development of an immune response to PEGylated drugs can result in the accelerated clearance of nanocarriers. For example, PEGylated liposomes lose their circulating properties in the 2nd week after systemic administration in mice (Hornung et al., 2008; Xitong & Xiaorong, 2016). It has been suggested that exosomes may have stealth properties that blunt their clearance by the immune system (Gibbings et al., 2009; Montecalvo et al., 2012; Mu et al., 2013). Exosomal drug formulations have been used to treat many diseases such as cancer, infection, and cardiovascular and neurodegenerative disorders (Xitong & Xiaorong, 2016). One of the first examples of the application of exosomes in drug delivery was in targeted delivery of the chemotherapeutic drug doxorubicin to mice with solid tumors. This study revealed a greater anti-tumor effect of targeted doxorubicin-encapsulated exosomes compared with free doxorubicin at equivalent doses (Tian et al., 2014). The superiority of exosomal doxorubicin has also been shown versus liposomal doxorubicin, a finding that has been attributed to the natural orientation of exosomal membrane proteins and their capacity for efficient interaction with the receptors in the target cell plasma membrane (Adlakha & Saini, 2014; Hornung et al., 2008; Xitong & Xiaorong, 2016). Apart from efficacy, intravenous administration is less toxic compared with the fee drug due to the specific accumulation of exosomes in the tumor tissue.

4 | NEUROLOGIC DISORDERS

Exosomes which are derived from mesenchymal stem cells are considered of therapeutic value for treating Alzheimer's disease (AD). Therefore, exosomes can be used in vivo as a vehicle to carry active neprilysin (NEP), the most important enzyme for β-amyloid (Aβ) peptide plug degradation in the brain (Sun et al., 2010). MSC-derived exosomes also decreased intracellular and extracellular Aβ levels in the neuroblastoma cell line N2A in vitro. It has been demonstrated that MSC-derived exosomes have a neuroprotective effect against stroke due, in part, to changing the miRNA profile of exosomes during the stroke, and then subsequently modifying the expression of miRNAs that participate in the recovery following a stroke (Wahlgren et al., 2012). Exosomal formulations of catalase is a more versatile strategy to treat inflammatory and degenerative disorders like Parkinson's disease (PD) (Kim, Bianco, Shufesky, Morelli, & Robbins, 2007).

5 | CARDIOVASCULAR DISORDERS

Exosomes from cardiosphere derived cells (CDCs) were shown to produce a range of different cardioprotective effects, such as anti-apoptotic, anti-inflammatory, anti-oxidative, anti-fibrotic, and cardiomyogenic effects (Jang & Gho, 2014; Pegtel et al., 2010). CDCs exosomes stimulated angiogenesis, induced cardiomyocyte proliferation, and reduced apoptosis in vitro. The capacity of these exosomes in regeneration was demonstrated in a chronic myocardial infarction model in rats and was attributed to the reduction of collagen deposition and inducing anti-fibrotic efficacy via paracrine mechanisms (Puri et al., 2009; Xitong & Xiaorong, 2016). Exosomes derived from endothelial cells were suggested to be a promising strategy to combat atherosclerosis, since atherosclerosis is the fundamental cause of myocardial infarction and stroke. Systemic administration of exosomes of human umbilical vein endothelial cells (HUVECs) reduced atherosclerotic lesions in mice fed with a high-fat diet. HUVEC exosomes were found to be enriched in multiple miRNAs resulting in controlled target gene expression and a reduction in atherosclerotic lesions of mouse aorta (Mu et al., 2013). In addition, exosomes derived from MSCs revealed drug-induced regeneration and cardioprotective paracrine effects against myocardial reperfusion or ischemia injury in liver-damaged mouse models, respectively (Ohno et al., 2013; Rechavi et al., 2009; van der Meel et al., 2014).

6 | PULMONARY DISORDERS

Hypoxia induces an inflammatory response in the lung by activation of macrophages with a subsequent elevation of proinflammatory mediators that may cause later development of hypoxic pulmonary hypertension (Lee et al., 2012). Pulmonary hypertension has also been reported to be treated by application of MSC-derived exosomes by suppression of early inflammation of lung and inducing vascular remodeling (Marcus & Leonard, 2013). Mesenchymal stromal cell-derived exosomes (MEX) suppress the hypoxic activation of signal transduction and activation of STAT3 and the upregulation of the miR-17 superfamily, whereas they increase lung levels of miR-204, which are decreased in human pulmonary hypertension. MEX produced by umbilical cord mesenchymal stromal cells inhibit STAT3 signaling in isolated human pulmonary arterial endothelial cells, showing a direct effect of MEX on hypoxic vascular cells. Therefore, MEX exerts a protective effect on the lung and inhibits pulmonary hypertension by suppression of hyperproliferative pathways (Lee et al., 2012).

7 | CANCER

Some studies have indicated that MSC-derived exosomes can be used as anti-cancer agents. In this regard, exosomes have a capacity of delivering drugs directly to the tumor microenvironment. MSC-derived exosomes loaded with Paclitaxel (PTX) have been reported to exert strong anti-cancer properties and could be tailored to be taken up and release their drug cargo in the tumor tissue (Barreto et al., 2010; Koppers-Lalic et al., 2013).

Exosomes with tumor antigens could stimulate CD4+ and CD8+ T cells resulting in inhibition of tumor growth (Yu & Finn, 2006; Zitvogel et al., 1998). For example, melanoma-derived exosomes contain the immunogenic antigens MelanA/Mart-1 and gp100, and CEA and HER2 are expressd by those released by colon carcinoma cells.

Exosomes derived from dendritic cells produce potent anti-tumor T-cell responses and tumor regression in experimental animals (Andre et al., 2002). Phase I clinical trials evaluated the effectiveness of patient-specific exosomes released by dendritic cells and loaded with tumor antigen peptides (Dexosomes [Dex]) for melanoma and non-small cell lung cancer. This trial demonstrated that dexosome immunotherapy is possible, safe, and involves both innate and adaptive immune responses, which resulted in stabilization of the disease and increased long-term survival for several patients (Delcayre & Le Pecq, 2006; Viaud et al., 2010).

Ascites derived exosomes from colorectal cancer patients were shown to be safe, nontoxic, and tolerable when used as a cancer vaccine, and, in association with GM-CSF, can efficiently induce potent carcinoembryonic antigen (CEA)-specific anti-tumor immunity in advanced colorectal cancer patients (Dai et al., 2008).

In cancer patients with advanced disease, tumor-derived exosomes do not exert any effective immune stimulatory or anti-tumor effects despite the production of tumor-derived exosomes (Zitvogel et al., 1998). Tumor-derived exosomes have also been immunosuppressive following direct administration, and have actually resulted in enhanced tumor growth (Delcayre & Le Pecq, 2006; Zitvogel et al., 1998). Tumor-derived exosomes were shown to either suppress the activity of effector T cells, or target myeloid cells, to modulate their differentiation and function, for example, in the case where exosomes are derived from human melanoma and colorectal carcinoma cell lines (Viaud et al., 2010; Zitvogel et al., 1998).

8 | EXOSOMES AS VACCINE CANDIDATES FOR INFECTIONS

Exosomes are selective candidates for use in vaccines for infections such as toxoplasmosis, diphtheria, tuberculosis, and atypical severe acute respiratory syndrome (SARS). It has been reported that transfer of DCs pulsed with *Toxoplasma gondii* antigens (TAg) to healthy mice induced protection against a virulent strain of *T. gondii* in an oral challenge, but it was difficult to obtain a sufficient quantity of DCs suitable for vaccination (Aline, Bout, Amigorena, Roingeard, & Dimier-Poisson, 2004; Beauvillain, Juste, Dion, Pierre, & Dimier-Poisson, 2009; Beauvillain, Ruiz, Guiton, Bout, & Dimier-Poisson, 2007).

Murine bone marrow-derived DCs pulsed in vitro with intact diphtheria toxin (DT)-released exosomes, which upon injection into mice, induce IgG2b and IgG2a responses specific for DT (Colino & Snapper, 2006). Infection with *Mycobacterium tuberculosis* stimulates macrophages to increase the release of exosomes and, it should be noted that microvesicles containing *M. tuberculosis* peptide-MHC-II complexes can produce antimicrobial T-cell responses (Ramachandra et al., 2010; Singh, LeMaire, Tan, Zeng, & Schorey, 2011).

Exosomes as a vaccine have also been explored in SARS-associated coronavirus infection which induces an atypical pulmonary

disease that can be fatal. Kuate, Cinatl, Doerr, and Überla (2007) demonstrated that exosomes containing spike S protein of SARS-CoV induced neutralizing antibody titers, which was further enhanced by priming with the SARS-S exosomal vaccine and then boosting with the currently used adenoviral vector vaccine. Exosomes may be also candidates as vaccines for allergic diseases. Exosome like vesicles isolated from the bronchoalveolar lavage fluid of mice by respiratory exposure to the olive pollen allergen induced tolerance and protection against allergic sensitization in mice (Prado et al., 2008).

9 | NEOVASCULAR DISEASES

Exosomes are being considered as a therapeutic tool in moderating neovascularization. Activation of neovascularization can lead to an increased healing of wounds. Additionally, reconstruction of hypoxic injury while preventing neovascularization, delays tumor development (Martinez & Andriantsitohaina, 2011). Exosomes which are secreted from human CD34+ cells have angiogenic activity in isolated endothelial cells and in murine models of vessel growth and represent a significant paracrine effect for therapeutic angiogenesis and enhancing recovery from injury or ischemic disease (Sahoo et al., 2011).

10 | AUTOIMMUNE DISEASES

Exosomes may also be useful in the treatment of autoimmune diseases in animal models. Kim et al. (2007) showed that the administration of exosomes derived from DCs expressing IL-4 were able to modify the activity of APC and T cells in vivo through a FasL/Fas-dependent mechanism, and resulted in an effective treatment against collagen-induced arthritis by suppression of a delayed-type

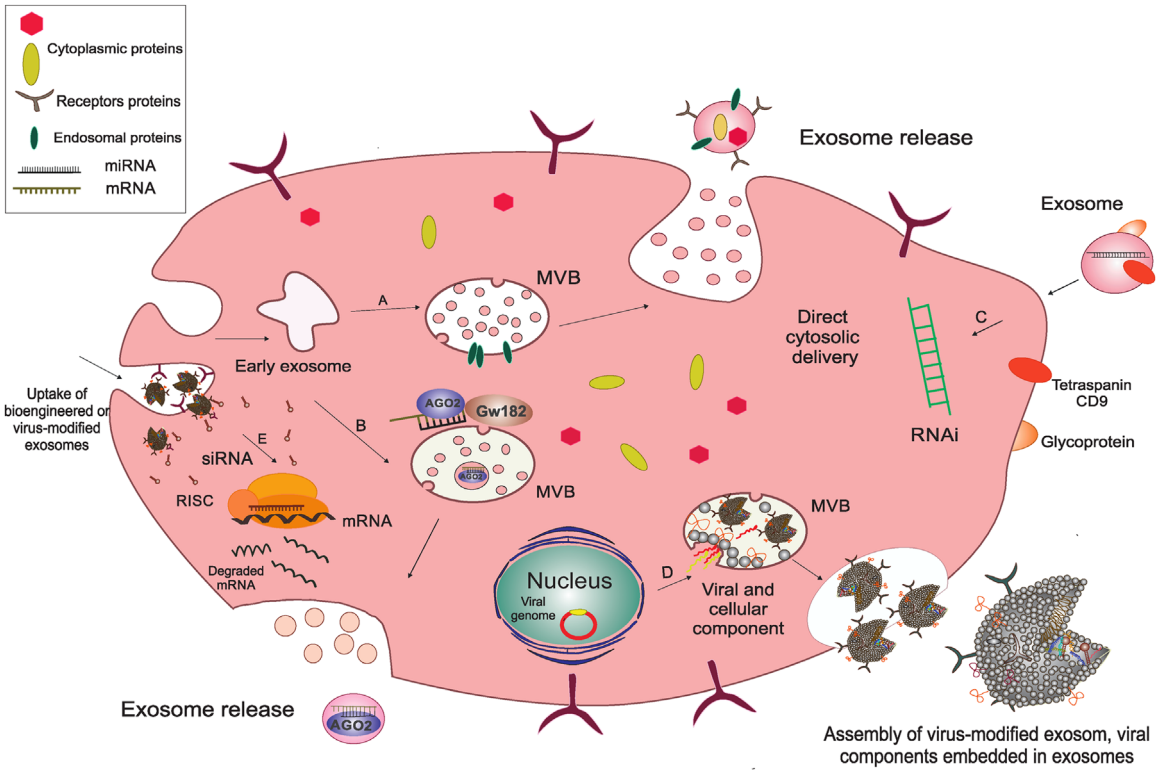

FIGURE 1 Schematic representation of the biogenesis of exosomes. Exosomes originate as endocytic vesicles through invagination of the cell membrane, which results in the formation of early exosomes and, subsequently, late exosome called MVBs. MVBs contain ILV which are formed from budding of the endosomal membrane and are called exosomes. When MVBs fuse with the plasma membrane, they give rise to the release of exosomes into the extracellular space. (A) Endosomal proteins such as CD9, Alix, and TSG101 may be incorporated into exosomes during their assembly to facilitate this process. (B) AGO2 and GW182 are two important components of the RNA-induced silencing complex (RISC), which associate with MVBs so as to mediate miRNA sorting into exosomes. (C) Subsequent fusion of cell-derived exosomes with the plasma membrane through their CD9 (tetraspanin) interaction with surface glycoproteins on target cells gives rise to cytosolic delivery of the siRNA directly. This process is involved in the creation of temporary RNAi. (D) Exosomes originate from inward budding in the lumen of the MVB through which the cytoplasmic content from the cell of origin and also viral components, including mRNA and small non-coding RNA and viral glycoproteins are incorporated into the exosome, and then released as a selective cargo in viral-infected cells. These virus-modified exosomes display the original surface markers and cell membrane as the parent cells. (E) Bioengineered or virus-modified exosomes are designed to express a selective set of proteins and small, non-coding RNAs to target specific receptors. Exosomes then fuse with the endosomal membrane to release their non-coding RNAs into the cytoplasm so as to load siRNA into the RNAi (RISC) complex of the target cells in order to prevent mRNA translation into protein. Acronyms: RISC, RNA-induced silencing complex; RNAi, RNA interference; siRNA, small interference RNA; MVBs, multivesicular bodies

hypersensitivity and inflammatory response. Also, vaccination of mice with exosomes from FasL, IL-10, and indoleamine 2,3-dioxygenase-modified DC decreased the clinical development of mice with rheumatoid arthritis (Kim et al., 2005, 2006; Kim, Kim, Oligino, & Robbins, 2002; Szántó et al., 2007; Yin, Ouyang, Li, Xiao, & Yang, 2013). Exosomes from TGF-β1-modified DCs decreased disease activity and occurrence of intestinal bleeding in a murine model of inflammatory bowel disease (IBD) (Cai et al., 2012; Yin et al., 2013).

11 | EXOSOMES AS DRUG CARRIERS

Exosomes should be able to carry a sufficient amount of therapeutic cargo to qualify as drug delivery vehicles. A variety of cargos exhibit desired therapeutic effects after exosome-based delivery to particular tissues (Johnsen et al., 2014). Exosomes are also known as a natural way of delivering various large-size proteins. For example, catalase has been incorporated into a nano-based polymer in order to preserve the therapeutic protein against degradation in host cells and improve loading capacity, and then subsequently loaded into exosomes (Beauvillain et al., 2009). Exosomal formulations of catalase are a more versatile strategy to treat inflammatory and degenerative disorders like Parkinson's disease (PD). Exosomes have been shown to be readily taken up by neuronal cells in vitro. In fact, a considerable quantity of exosomes was detected in the brains of mice with experimentally induced Parkinson's disease following intranasal administration of catalase-loaded exosomes. The catalase-loaded exosomes provided neuroprotective effects in both in vitro and in vivo models of PD (Haney et al., 2015). Different strategies have been considered in an attempt to load proteins and other biomolecules into exosomes, including incubation at room temperature, exposure to freeze-thaw cycles, permeabilization with saponin, sonication, or extrusion (Kuate et al., 2007; Prado et al., 2008; Singh et al., 2011). Murine MSC-secreted exosomes were loaded with paclitaxel (PTX) by incubating the parent cells with the drug. Results showed a significant amount of PTX was loaded into the exosomes as demonstrated by HPLC (Beauvillain et al., 2007). A similar result was reported for HepG2 cells that were incubated with different anti-cancer agents such as PTX, etoposide, carboplatin, irinotecan, epirubicin, and mitoxantrone; all of which, resulted in anti-proliferative activity (Lv et al., 2012).

12 | CONCLUSION

To summarize, exosomes are naturally occurring nanovesicular structures that are secreted by almost all cell types in all body fluids. Their function in cell–cell communication between distant neighboring cells caused researchers to utilize exosomes as a "next generation" carrier for gene therapy. Moreover, exosomes have many more advantages when compared to all other existing delivery systems, including the fact that they are nanosized vesicles, they exhibit permeability of biological membranes, they have limited safety concerns, as well as immunological inertness, non-mutagenesis, immunomodulatory and regenerative properties, and protein orientation similar to the original cells from which they were derived. Being composed of not only lipid and protein, but also nucleic acids (especially various RNAs), makes exosomes suitable as potential carriers for exogenous cargos, including RNAi and other therapeutic compounds (Fig. 1). Many obstacles associated with RNAi (miRNAs and siRNAs) delivery in vivo have been overcome by exosome-mediated delivery systems. Furthermore, exosomes are capable of delivering exogenous therapeutic agents to a specific tissue. Alvarez and others demonstrated successful targeted RVG-exosome delivery to the brain.

Exosomes are considered a promising strategy for effective and safe drug delivery to target cells. To accelerate the progress toward the routine use of exosomes for gene delivery, several issues need to be addressed. For example, new and improved technology is critically needed to efficiently load therapeutic agents into exosomes. Additionally, obtaining highly purified exosomes in large quantities still requires further investigation. Indeed, the efficiency and safety features of exosome-based drug formulations must be rigorously compared to existing gene delivery systems to maximally exploit this approach for treating life-threatening diseases. In conclusion, with continued technological advancements, exosome-mediated delivery of drugs, genes, and other biotherapeutics will be transitioned from bench research to a clinical setting.

CONFLICTS OF INTEREST

The authors have no disclosures or other conflicts of interest to report.

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