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Exosomes as a Drug Delivery System in Cancer Therapy: Potential and Challenges

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Abstract

Exosomes play a pivotal role in mediating intercellular communications and package delivery. They have recently been discovered to serve as diagnostic biomarkers as well as a possible drug delivery vehicle based on their nanometer size range and capability to transfer biological materials to recipient cells. Their unique biocompatibility, high stability, preferred tumor homing, and adjustable targeting efficiency can make exosomes an attractive and potentially effective tool of drug delivery in cancer therapy. While exosomes possess properties that make them uniquely suitable for delivery of bioactive molecules, there remains a to-be-filled gap between the current understanding about exosome biology and the ideal application scenarios. In this review, we summarize the characteristics enabling the potential of exosomes for drug delivery as well as the outstanding questions related to exosome composition and function, production and purification, bioengineering and targeting, uptake and biodistribution, efficacy and immune regulation, etc. Advanced technologies are demanded to visualize, characterize, and sort heterogeneous exosome populations. We are positive that the deeper and more comprehensive understanding of exosome biology as well as advanced nanotechnology will certainly accelerate its therapeutic applications.

Graphical Abstract

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Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.molpharmaceut.8b00277. Schematic representation of the critical factors impacting the application of exosomes as a nano-drug delivery vehicle and a schematic of an exosome (PDF)

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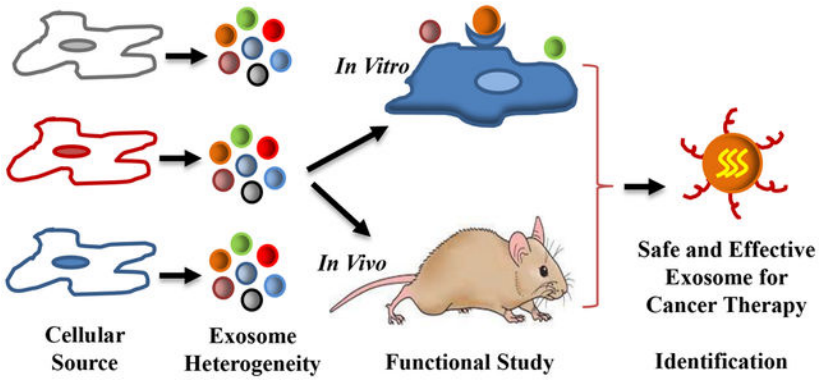

Keywords

exosomes; drug delivery system; biodistribution; tumor homing

INTRODUCTION

Exosomes, small extracellular vesicles (EVs) of nanoscale size (30–100 nm), are secreted by various cells.[1–3] The past decade has witnessed a fast-growing research interest in exosomes and other EVs, mostly due to the identification of different functional molecules EVs carry and the pathophysiological role they play. The inherent ability of exosomes to transfer materials between cells makes them appealing as natural drug delivery vehicles. In fact, several groups have already utilized these vesicles for the delivery of siRNAs,[4,5] miRNAs,[6,7] and shRNAs,[8] as well as the anti-inflammatory agent curcumin[9,10] and the anticancer agents doxorubicin[11] and paclitaxel,[12] into animal models. Recent studies demonstrated that compared to other delivery systems, exosomes can greatly increase the loading efficacy of doxorubicin and significantly decrease the adverse effects on major organ systems, especially the heart, suggesting that drug delivery via exosomes may alleviate the major shortcomings of their chemotherapeutic cargo.[11,13]

To exploit the opportunities for utilizing exosomes as a drug delivery vehicle in cancer therapy, a comprehensive evaluation of exosome properties is necessary, including the vesicular size, source, surface properties, targeting strategies, cellular trafficking and fate, in vivo biodistribution, clearance, and tumor homing efficiency. As exosomes are secreted by various cells and circulate in many body fluids, informative evaluations include their half-life in circulation, molecular properties, and stability in circulation and in biological fluids, such as blood, urine, saliva, milk, and pleural effusions. To better understand and evaluate the capacity, suitability, and efficacy of exosomes as a drug delivery system, several criteria should be addressed, as highlighted in Figure 1.

COMPOSITION OF EXOSOMES

Among various separation technologies, including ultrafiltration and immunoprecipitation, ultracentrifugation is being widely used for the isolation and purification of exosomes.[3,14] The composition and yield of exosomes differ depending on the parent cells, physiological

conditions, and environmental stimulation. Simply put, exosomes are a small volume of cytosol enclosed by a lipid membrane. However, the new found complexity and pathophysiological impact of exosomes has generated a lot of interest around their biological makeup. According to the exosome content database (Exocarta), exosomes from various organisms and cell types contain 4563 proteins, 194 lipids, 1639 mRNAs, and 764 miRNAs.[15,16]

Biochemical and proteomic analyses of exosomes have revealed the presence of lipids, proteins, nucleic acids and other components (Figure 2), where lipids are believed to be the main component. For example, cholesterol, diglycerides, phospholipids, sphingolipids, glycerophospholipids, and ceramides are enriched in exosomes compared to the parent cells.[17–19] In addition to these lipids, several bioactive lipids, such as prostaglandins and leukotrienes, and enzymes (e.g., phospholipase C) are also found in exosomes.[17,20] The protein content largely depends on the exosome's cellular origin and is generally enriched in certain molecules, including targeting and fusion proteins (e.g., tetraspanins, lactadherin, and integrins), cytoplasmic enzymes (e.g., GAPDH, peroxidases, pyruvate kinases, and lactate dehydrogenase), chaperones (e.g., heat shock proteins Hsp60, Hsp70, and Hsp90), membrane trafficking proteins (e.g., Rab proteins, ARF GTPases, and annexins), proteins involved in multivesicular body (MVB) formation (e.g., ALIX, TSG101, and clathrin), cytoskeletal proteins (e.g., actin and tubulin), and signal transduction proteins (e.g., protein kinases and heterotrimeric G proteins).[21] Other key proteins include tetraspanins CD9, CD63, and CD81/CD82; the flotillins (Flot1 and Flot2); different Rab-GTPases or Tsg101 of the ESCRT I complex; cytoskeleton proteins (e.g., β-actin, moesin, cofilin, and tubulins); and major histocompatibility complex (MHC) class I and class II molecules (Figure 2).[22] Furthermore, exosomes also contain proteins involved in cell signaling pathways, such as the Notch ligand δ-like 4,[23] Wnt-β-catenin signaling proteins,[24] and some proteins involved in intercellular signaling, such as interleukins.[25] Hsp70 and CD63 are the most frequently recognized proteins in exosomes. Tetraspanins CD9, CD63, and CD81 are involved in exosome biogenesis and protein loading.[26,27] In addition to proteins and lipids, exosomes also contain functional RNA molecules, including mRNAs and other noncoding RNAs, such as miRNAs and lncRNAs.[2,28]

FUNCTION OF EXOSOMES

Exosomes mediate normal physiological functions through cell−cell communication by transferring functional molecules present on their surface and in their lumen (miRNA, mRNA, lipid, proteins, various noncoding RNAs, mitochondrial DNA, genomic DNA, etc.) from one cell to another.[2,29,30] The surface of a nanoscale drug delivery system mediates direct contact with the recipient cell surface, and thus plays the initial and foremost role in cellular recognition and internalization both in vitro and in vivo. Compared to synthetic nanoparticles, exosomes as natural products are more compatible with biological systems. In addition, exosomes can regulate the gene expression of recipient cells via the delivery of specific mRNAs and miRs.[2,31] However, depending on their cellular origin and the bioactive molecules they contain, exosomes can play dual roles in regulating tumors. On the one hand, exosomes from mature dendritic cells can inhibit tumor growth.[32] Excitingly, exosomal content, such as the fatty acid docosahexaenoic acid and lysophosphatidylcholine, can

enhance dendritic cells' antigenic capacity.[33] On the other hand, exosomes isolated from cancer cells significantly promote tumor growth in mice,[34] possibly by delivery of tumorigenic miRNAs.[35–37] Similarly, exosomes containing high levels of prostaglandin PGE2 and TGF-β promote tumor immune evasion and tumor growth.[38] Exosomes from colorectal cancer contain mRNAs that enhance endothelial proliferation, thereby inducing tumor angiogenesis.[39] Moreover, proteins, mRNA, and sphingomyelin present in glioblastoma-derived exosomes promote tumor angiogenesis, tumor invasiveness, and vessel lumen formation.[40] Therefore, depending on the goals of drug delivery, it is critical to identify the most suitable exosome-producing sources (cell lines) and avoid any tumor-promoting side effects.

SOURCES OF EXOSOMES

Exosomes exist and can be produced from a wide variety of sources, such as cell culture supernatant, plasma, serum, urine, milk, or other biofluids.[14] Nevertheless, exosomes are also heterogeneous in size, have a surface molecule composition and placement, and have the ability to carry various cargoes. Furthermore, the presence of bioactive surface molecules on exosomes correlates with their cellular origin. For example, antigen-presenting cell-derived exosomes are enriched in antigen-presenting molecules, including MHC-I and -II complexes, as well as co-stimulatory molecules.[41] Tumor-derived exosomes usually contain tumor antigens in addition to certain immunosuppressive proteins, such as FasL, TRAIL, or TGF-β.[42] Thus, therapeutic exosomes must be produced from selective normal cells with desired properties and carefully purified to avoid contamination of undesired molecules. Development of advanced technologies to sort subsets of exosomes and EVs by size and surface properties would be useful to increase purity, reproducibility, and efficiency of obtaining uniform exosome samples. Prior to purification, it is necessary to improve the yield of desired exosomes from large volumes of cell culture supernatants or biofluids. One of the new approaches to efficiently produce safe-to-use exosomes is using the Integra CELLine Culture System.[43] The Integra CELLine Culture system includes a two-compartment flask with a semipermeable membrane that surrounds a concentrated cell-containing compartment.[43] This allows a constant collection of secreted exosomes, which flow out from the inside cell-containing compartment as well as a continued refilling of nutrients which flow back in from the larger, outer medium compartment by collecting and replacing the outer medium.

PURIFICATION AND CHARACTERIZATION OF EXOSOMES

In addition to the sources and yield of exosomes, purification of exosomes remains another potential challenge to their future application in cancer therapy.

With rapid advancement in science and technology, several methods, including differential ultracentrifugation[14] and density gradient ultracentrifugation (sucrose density gradients or sucrose cushions),[14] size-based isolation (ultrafiltration),[14,44] exosome precipitation[45] (www.systembio.com), immunoaffinity-based capture of exosomes,[46] and microfluidics-based isolation,[47] have been developed for the isolation and purification of exosomes and exosomal RNAs. The features of exosomes, such as their density, shape, size, surface

proteins, biochemical composition etc., may vary depending on the method of isolation. Moreover, the isolation methods may have an impact on quality, quantity, and physicochemical properties of exosomes.

To examine the quality and quantity of exosomes, several methods have been developed to evaluate their size, size distribution, shape, surface charge, quantity, and biochemical composition, including (1) superhigh resolution electron microscopy (EM), cryo-EM, and immuno-cryoEM to assess the structure, size, and surface molecules; (2) low resolution nanoparticle tracking analysis (NTA)[48] to reveal the size and physical features like the zeta potential; (3) protein analysis via mass spectrometry, Western blotting, immunofluorescence staining, and ELISA; (4) RNA analysis via RNA sequencing, PCR, and other platforms; and (5) biochemical analyses of lipids, sugar, and other components. Isolated exosomes can be validated with antibodies against exosome markers, such as CD9, CD63, CD81, and TSG101, using a confocal microscope, Western blot, conventional flow cytometer, or advanced micro flow cytometer, as we recently developed.[3,14]

A careful evaluation is necessary to optimize the isolation and purification method of exosomes for their application in drug delivery.

LABELING OF EXOSOMES

Purified exosomes can be labeled using fluorophores for qualitative and quantitative evaluation of cellular targeting, uptake, internalization routes, trafficking in cells, and biodistribution and tissue accumulation in vivo using fluorescence microscopy. The presence of a lipid membrane around the perimeter of exosomes allows for the binding of fluorescent lipophilic dyes, like PKH67,[49,50] PKH26,[51] rhodamine B (also known as R18),[52] DiI,[52,53] DiD,[40] and DiR.[7] Membrane-permeable chemical compounds, such as carboxy-fluorescein succinimidyl ester (CFSE) and 5(6)-carboxyfluorescein diacetate (CFDA), can also be used to stain exosomes. These compounds enter into exosomes and, once confined to the cytosolic lumen, are exposed to fluoresce as a consequence of esterification.[53,54] For the quantitative evaluation of in vivo biodistribution and tissue accumulation, exosomes can be labeled with radioisotopes ([^{111}Indium]-oxine[55] or [3H]-CHE, which are commonly used to label the synthetic nano-particles[56]). To visualize the biodistribution and clearance of exosomes and other EVs in vivo, an elegant EV imaging reporter system has been developed.[57] Furthermore, exosomes can be intrinsically labeled during their biogenesis by transducing the cells with membrane-bound fusion proteins tagged with GFP/RFP[58] or other fluorescent proteins.[59] Another labeling approach was also developed where purified exosomes were reacted with carboxytetramethylrhodamine succinimidyl ester (TAMRA-NHS).[60] However, further evaluation is necessary to evaluate whether the integrity of exosome surface molecules and biological behavior is compromised after such labeling.

SURFACE MOLECULES AND BIOENGINEERING OF EXOSOMES

One of the goals of exosome bioengineering is the development of targeted exosomes for cancer therapy. The basic idea of this approach is to place a specific targeting molecule (peptides or other ligands) on the surface of exosomes that mediates the interaction with and

subsequent internalization into the desired cell type (Figure 2). The main challenge to this approach is the fact that many as-yet uncharacterized surface molecules and cellular components of exosomes may control their internalization and fate upon uptake. A complete understanding of the fundamental biology of exosome biogenesis, trafficking, and internalization is yet to be achieved, and exosome heterogeneity needs to be better appreciated.

Several studies have demonstrated the feasibility of targeting specific tissues or cell types using engineered EVs. For example, exosomes have been biologically engineered to express surface proteins fused to customized peptides.[61] The engineered expression of brain-specific rabies viral glycoprotein (RVG) or internalizing RGD peptide (CRGDKGPDC) with an EV membrane protein lysosome-associated membrane glycoprotein 2b (Lamp2b) on the surface of EVs enhanced targeting and drug delivery efficiency to the brain.[61,62] Furthermore, expression of GE11 peptides fused to transmembrane domains of platelet-derived growth factor receptor on EVs exhibited specificity for epidermal growth factor receptor (EGFR)-expressing tumors.[7] Together, these data highlight the possibility of engineering EVs for specific tissue targeting and drug delivery.

DRUG LOADING INTO EXOSOMES

Exosomes contain proteins, lipids, and various other bio-molecules in their lumen. Therefore, efficient loading of external drugs or molecules into exosomes is another demanding and challenging task. Like synthetic nanoparticles, several methods, including direct mixing, incubation, sonication, vortexing, remote loading, electroporation, and transfection, can be applied to load micro- and macromolecules into exosomes. For some hydrophobic drugs (e.g., curcumin), EVs can be loaded with the drugs by direct mixing.[46] Paclitaxel can be loaded by mixing and sonication.[47,48] Due to the presence of the lipid bilayer around the exosome perimeter, electroporation is widely applied to load nucleic acids (siRNAs).[19,49–51]

Although the loading of drugs or macromolecules into exosomes/EVs has been achieved in many cases, it remains unclear whether these loading strategies disrupt the integrity, stability, function, and loading/retention efficiency of the vesicles. Previous reports suggest that electroporation may cause aggregation or fusion of the vesicles.[63] Therefore, multiple approaches toward loading and quality assessment are essential to evaluate the above properties of drug-loaded vesicles to ensure their functionality and efficient delivery of therapeutics to the target sites.

CELLULAR TARGETING, UPTAKE, AND INTRACELLULAR TRAFFICKING

The ability of exosomes to transfer biochemical content from donor cells to recipient cells makes them attractive candidates for application in drug delivery. Like chemically engineered nanoparticles, exosomes are also internalized into cells in a cell type-dependent manner[64] via membrane fusion,[65,66] clathrin-mediated endocytosis,[67] lipid raft-mediated endocytosis,[68] heparin sulfate proteoglycans-dependent endocytosis,[50] phagocytosis,[64] and ligand−receptor interaction.[69] The mechanisms of EV uptake and content delivery (or

degradation) vary among EV types and recipient cell types. However, very little is known about the relationship between size, surface chemistry, internalization, and intracellular trafficking of exosomes. Pending tasks include elucidating and understanding the cellular trafficking, endosomal escape, lysosomal degradation, and drug delivery efficiency of exosomes at the high-resolution subcellular level as well as the global, comprehensive in vivo landscape.

Entry of EVs into recipient cells can be measured and monitored using flow cytometry and confocal microscopy. To distinguish between internalized and surface-bound fluorescent EVs, the surface of the cell can be stripped by treatment with acid,[64] trypsin,[70] or heparin, which is being used for nanoparticles.[71] Such studies suggest that many cells do indeed internalize EVs. Employing real-time fluorescence microscopy and a single-particle tracking system allows us to better understand the motion of exosomes at the plasma membrane and distinguish endocytosis from fusion during exosome uptake. Additionally, these new tools provide insight into the exosome-cell interaction mode and the intercellular trafficking of exosomes, to understand the roles of exosomes at cellular level.[52] Furthermore, Rogers et al. reported a new particle trafficking software algorithm (PolyParticleTracker) to accurately track endogenous particles of different sizes in cells.[72] These approaches will assist us to understand the intracellular trafficking of exosomes in the subcellular compartments, providing a fundamental premise for harnessing exosomes as therapeutic delivery vehicles.

BIODISTRIBUTION OF EXOSOMES

The in vivo biodistribution of exosomes and their ability to target specific cells or tissues will determine their suitability for use as therapeutic tools or drug delivery systems. In particular, the size and surface of nanoparticles not only govern their intertissue biodistribution and systemic stability but also determine their intratissue distribution (e.g., inside tumors).[56] Excitingly, exosomes show potential as an advantageous drug delivery system in cancer therapy based on their size, presence of lipid bilayer, surface property, and the cargos they carry (Figure 2). Once the targeting specificity of engineered exosomes is achieved in vitro, the next important step is to evaluate their biodistribution features in vivo.

A few studies have examined the biodistribution of EVs in vivo. Wiklander et al. evaluated the biodistribution of DiR-labeled EVs from various cell sources. They found that while the highest accumulation of EVs occurs in the liver, spleen, lung, and gastrointestinal tract at 24 h after intravenous (i.v.) injection into mice, EV parent cells can influence the biodistribution of EVs.[73] In another study, Lai et al. reported that the highest bioluminescent signals of Gaussia luciferase (Gluc)-labeled EVs were observed in liver and spleen, from which the EVs were cleared 6 h after i.v. administration in athymic nude mice.[57] Recently, Smyth et al. reported similar biodistribution profiles and clearance rates between labeled EVs, synthetic liposomes, and the liposomes made from lipid extracts of EVs.[55] In addition, Bala et al. found that, upon i.v. administration of miRNA-loaded EVs into miRNA-155$^{-/-}$ mice, the highest miRNA-155 signals were observed in the liver, followed by adipose tissue and lungs, and the lowest signals were observed in muscle and kidneys.[74] Together, these reports demonstrate that systemically administered EVs are cleared by the mononuclear

phagocyte system (MPS), particularly in the liver and spleen, which is reflected in the relatively short half-life in the systemic circulation.

In contrast, the patterns of biostability and clearance of unmodified exosomes resemble those of synthetic nano-particles, such as non-PEGylated liposomes. A recent study showed that exosomes exhibited enhanced retention in the circulation, likely due to the CD47-mediated protection of exosomes from the MPS,[75] and were able to facilitate tumor targeting in mice. Nevertheless, the enhanced stability and tumor-targeting specificity of exosomes over synthetic nano-particles has yet to be optimized, and to obtain specific tumor-targeting exosomes, bioengineering and modifications of exosomes will be necessary.

It is well documented that PEGylated or targeted nano-particles, at ~100 nm in size, home to tumor tissues via a novel mechanism called the enhanced permeation and retention (EPR) effect.[76,77] Since exosomes are equally small, within the range of ~100 nm, it is predicted that upon i.v. administration, exosomes will also be able to home to tumor tissues following the EPR mechanism. However, it is yet to be elucidated if exosomes have additional inherent mechanisms other than the surface display of CD47 or mimicking PEGylation to protect them from being recognized by the MPS and filtered/cleared out of the systemic circulation. To increase their circulation time and to make them more biostable, PEGylation of exosomes has recently been applied (Figure 2). Non-PEGylated EVs have been observed to rapidly clear from the circulation within 10 min after i.v. injection in mice, whereas PEGylation increases their circulation time up to 60 min.[78] While this approach seems promising, an extended clearance time of >60 min may be necessary to improve the biostability of exosomes. Further studies are necessary to develop EVs with prolonged half-lives and increased vascular permeability to improve targeting to specific tissues and tumors. Finally, it may depend on the cellular origin of exosomes, surface modifications, and the route of administration.

TUMOR HOMING PROPERTIES OF EXOSOMES

The biggest advantage of nanoscale drug delivery systems for cancer therapy is the leaky blood vessels of tumors, with a selective accumulation of nanosize particles and vesicles via the EPR effect.[76,77] However, the vascular leakiness varies in a tumor type-dependent manner, so the size of the nanodrug delivery vehicle needs to be customized to match the tumor vasculature. Ideally, exosomes at certain sizes within the range of 30–100 nm would be sorted or purified (Figure 2), thereby enabling penetration of and accumulation in a specific type of tumor. While considerable effort has been expended in search of exosome isolation and characterization methods, as well as the development of engineered exosomes, specific targeting and high efficacy remain to be huge obstacles to the therapeutic application of exosomes.

EXOSOME IN IMMUNOTHERAPY

Raposo et al. first demonstrated the potential of exosomes as T cell primers more than 20 years ago, when they revealed B cell-derived exosomes could induce antigen-specific MHC class II restricted T cell responses.[22] Two years later, Zitvogel et al. further demonstrated

how dendritic cell (DC)-derived exosomes pulsed with tumor-derived peptides could stimulate significant antitumor T cell responses in tumor-bearing mice, proposing the use of exosomes as future tumor vaccines.[79,80] Since then, exosome-based immunotherapies have been conducted in various cancers, such as melanoma, glioma, hepatocellular carcinoma, and renal cell carcinoma.[81–85]

The rising interest in using exosomes as tumor vaccines is due to their ability to transfer antigens from professional antigen presenting cells (APCs), such as DCs, to other APCs, amplifying antigen-specific immune responses.[86] Exosomes derived from DCs express high levels of co-stimulatory molecules, such as CD40, CD80, and CD86, which likely promote these immune stimulating effects.[87] Furthermore, studies have established the superiority of exosomes over traditional tumor cell lysates, including efficient DC uptake and extended antigen storage, presentation, and processing.[88–90] In fact, previous work from Damo et al. showed exosomes derived from DCs loaded with B16F10 melanoma cell lysate and matured with TLR-3 ligand effectively stimulated recruitment of melanoma-specific CD8+ T cells, NK cells, and NK-T cells.[91] As a result, they observed reduced tumor growth and better survival compared to DC-derived exosome vaccines similar to those previously tested in human patients.[91,92] Consistently, similar effects are observed in a study of cervical cancer treatment in mice.[93]

Tumor-derived exosomes have also been tested as anticancer vaccines in vivo. Similar to DC-derived exosomes, the use of tumor cell-derived exosomes is appealing due to their ability to internalize into other cells. Additionally, tumor cell-derived exosomes are enriched in surface proteins that facilitate intercellular interactions. Some surface proteins include MHC I, which is essential for antigenic peptide capture, and HSP70, which is a critical chaperone for binding with DCs.[94] Furthermore, tumor cell-derived exosomes are also enriched in tumor antigens, such as NY-ESO-1 and MAGE-1, contributing to antigen presentation.[79] For example, leukemia cell-derived exosomes are observed to target DCs to stimulate potent anticancer immunity in mice.[95] Co-administration of glioblastoma (GBM) cell-derived exosomes and DCs pulsed with the invariant natural killer T cell agonist, α-galactosylcer-amide (α-GalCer), also induced a strong antigen specific cytotoxic T cell response against GBM in rats.[96]

While the use of exosomes as tumor vaccines have generated promising anticancer effects in vivo, clinical trials administering exosomes have not been as successful. Previous clinical studies include two Phase 1 clinical trials, which administered DC-derived exosomes loaded with tumor-associated antigens to advanced melanoma and nonsmall cell lung cancer (NSCLC) patients,[92,97] as well as one Phase II clinical trial, which administered interferon γ (IFN γ)-stimulated DC-derived exosomes to NSCLC patients.[98] While these studies demonstrated partial immunological and clinical responses and minimal toxicity in patients, survival benefits were largely variable and limited.[92,97,98] Interestingly, new approaches in ongoing clinical studies are administering plant-, such as grape, derived exosomes to colon cancer patients in combination therapies due to their ability to strongly bind hydrophobic drugs (including curcumin) and efficiently internalize into intestinal cells, including resident immune cells.

The use of exosomes as immunotherapies has greatly progressed throughout the past 20 years. While the growing feasibility of efficient RNA sequencing technologies and the trend toward personalized medicine may foster next generation exosome tumor vaccines by utilizing mutated antigens identified at individual levels, we are still far from effectively developing these vaccines as treatment and/or prevention. Further studies in exosome biology and engineering can continue to stimulate novel approaches necessary to reap the benefits of exosome anticancer immunotherapies.

COMPARISON OF EXOSOMES WITH SYNTHETIC NANOPARTICLES

A close comparison of biological features between exosomes and synthetic nanoparticles helps us weigh their respective advantages and disadvantages. A couple of synthetic nanoparticles have been studied in cancer therapy and approved by the FDA in the clinical delivery of chemotherapeutics.[99–101]

Synthetic nanoparticles are easy to manufacture, allowing for advantageous simplicity in isolation and purification as well as a high yield. However, the high toxicity and lack of targeting specificity have hindered their wide application. The most promising advantage of exosomes is their biocompatibility and minimal toxicity if they are produced by normal cells. While the synthetic nanoparticle field is relatively young, exosomes as a drug delivery system are still in the infancy stage. There are very limited studies comparing both in parallel; however, a recent study reported advantageous tumor targeting and efficacy of exosomes versus liposomes.[75] The remaining question is if the liposomes used in the study were clinically approved liposomes. There are different types of liposomes (anionic, cationic, PEGylated, ligand modified, pH sensitive, etc.), and a comprehensive comparison between all of these types of liposomes with exosomes might be necessary to establish the superiority of exosomes.

Drug loading efficiency is another parameter to compare between exosomes and synthetic nanoparticles. Since exosomes are inherently loaded and packed with various molecules during their biogenesis process (Figure 2),[15,16] it may be challenging to achieve high drug loading efficiency,[102] as compared to the unloaded synthetic nanoparticles.[71] Nevertheless, the drug loading efficiency of exosomes can be possibly improved through both external and internal loading approaches, such as electroporation as well as intrinsic loading and a biogenesis process.[103] Upon a better understanding of exosome bio-genesis, exosomes may be engineered to load with specific cargoes of interest through biological approaches, such as selective packaging in the exosome-producing cells.

In terms of biostability and tumor targeting, both synthetic nanoparticles and exosomes have a lot of room for improvement. The potential of exosomes relies on their naturally occurring surface membrane and bioengineering capacity. For example, a recent study demonstrated that exosomes harboring a signal regulatory protein α (SIRPα) on their surface can escape from being phagocytosed by macrophages,[104] thereby exhibiting biostability and a long circulation time. While peptides or targeting molecules loaded in synthetic particles very often lose their conformation and binding activity, we speculate that the bioengineered surface molecules expressed on the bilayer lipid membranes of exosomes would be more

biologically compatible and active (Figure 2). For example, since RGD is the most commonly used peptide to modify liposomes, it may be overexpressed and presented in one of the targeting molecules of exosomes with a much higher binding specificity and affinity to targeted tumor cells compared to liposomes. However, the uncharacterized complexity of exosomes could be one of the barriers to be overcome.

CONCLUSIONS

In this review, we particularly evaluate the biological features of exosomes that are critical for drug delivery, including composition, function, sources, purification and characterization, labeling, surface molecules and bioengineering, drug loading, cellular targeting, uptake, and intracellular trafficking, biodistribution, tumor homing properties, exosome in immunotherapy, and the comparison of exosomes with synthetic nanoparticles as drug delivery systems. While the utilization of exosomes for therapeutic drug delivery is still in its infancy, a more advanced understanding and systemic evaluation of the above issues will boost the development of exosomes as a superior and effective drug delivery system that can bring breakthroughs to the field of cancer nanomedicine and immunotherapy.

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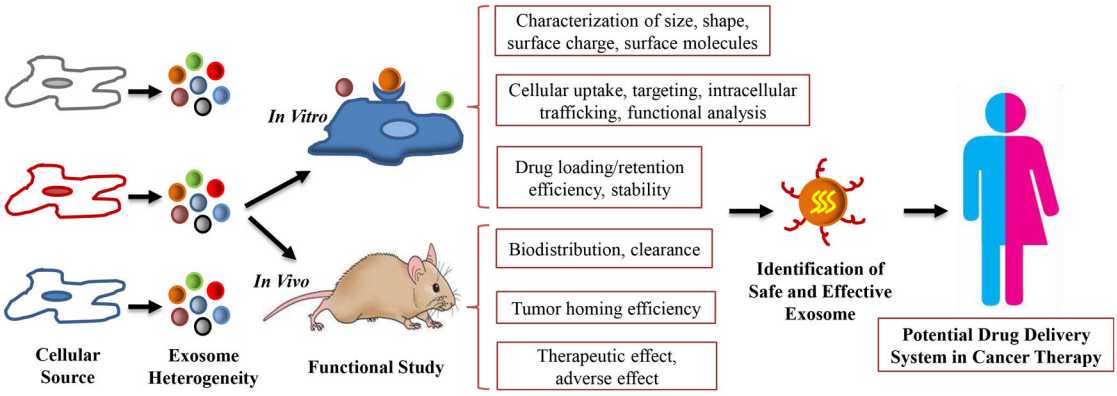

Figure 1.

Schematic representation of the critical factors impacting the application of exosomes as a nanodrug delivery vehicle.

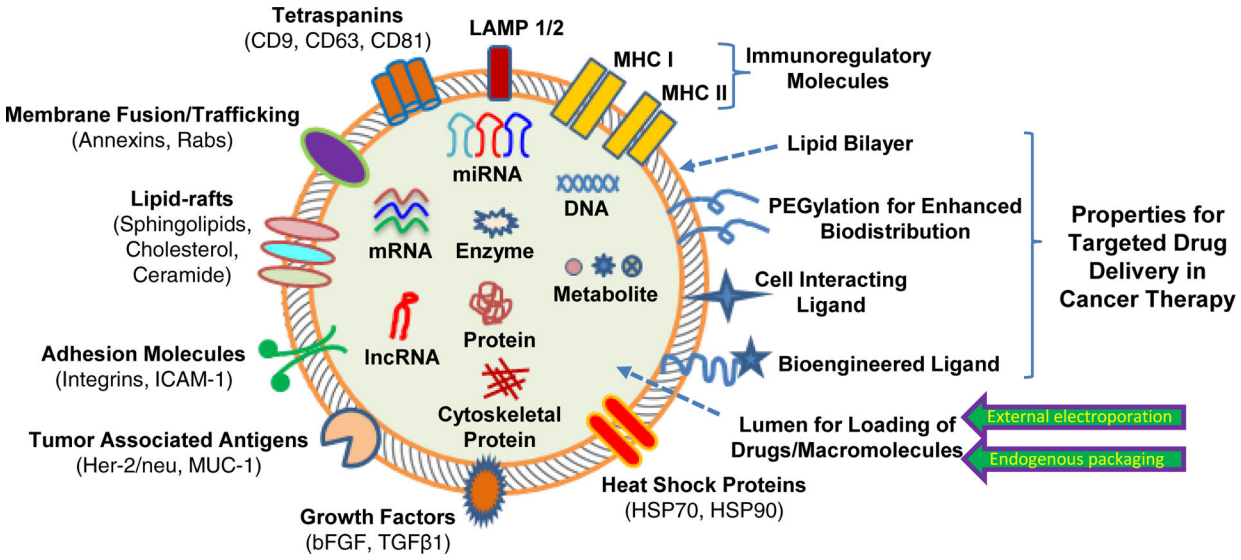

Figure 2.

Schematic of an exosome. Exosomes are cell secreted vesicles of ~100 nm in size and packed with a variety of cellular components, including mRNAs, miRNAs, proteins, enzymes, lipids, carbohydrates, etc. The exosome surface is decorated with various membrane proteins responsible for different pathophysiological functions. The size, lipid bilayer, surface molecules, incorporation of PEGylation, expression of specific ligands via bioengineering, as well as the sorting of a targeted exosome group from heterogeneous populations will collectively make exosomes an effective drug delivery tool in cancer therapy.