REVIEW PAPER

Exosomes as delivery tools in cancer therapy: Future perspectives

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ABSTRACT

Exosomes, which are one of the extracellular vesicles, are considered necessary tools of intracellular communication that abundant in our body in physiological and pathological conditions with a diameter of 30-150 nm. The nanotubes, dendrimeric, metallic, nanoparticles have been used in the medical area. However, these nano-based systems are lack of standardized manufacturing methods and therefore, it has toxic effects on cells. The delivery methods of growth factors, exosomes, cells, and engineered tissues have notably advanced in the medical area. The fact that they contain bioactive molecules such as protein, lipid, RNA and DNA revealed that these structures may play an important role in the treatment of cancer. Here, we review work on the contribution exosomal mediated cancer treatment in two main topics as exogenous molecule carrier and self-use. We also emphasize the development of exosome-based systems by referring to the advantages and disadvantages of using exosomes and future perspectives in cancer therapy.

Keywords: *Advantages, Cancer treatment, Cancer diagnosis, Disadvantages Exosome, Nanoparticle*

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INTRODUCTION

Nanoparticles are widely used systems for controlled release and targeted delivery. The metallic, polymeric, carbon-based, lipid-based nanoparticles, etc., have a great potential in imaging or therapeutic drug delivery [1-6]. However, these synthesized nanoparticles have cytotoxicity, loss of targeting capacity, and rapid clearance by the reticuloendothelial system (RES) [7-12]. By contrast, exosomes are small nanovesicular carriers that transport cargo and they are related to the pathology of diseases. Furthermore, they can passage the blood-brain barrier (BBB) for brain-targeted drug delivery [13, 14]. Exosomes have drug loading and signal-carrying capacity; these properties of them have shown great potential in recent years. Nowadays, the use of exosomes as targeted therapeutical carriers have increased in the literature. Dhayapulay and Kanapathipillai prepared heat shock protein 90 (HSP90) inhibitor geldanamycin-loaded exosomes and applied it to cancer cells. Therefore, exosomes can be carrier transport therapeutic drugs and used to treat diseases such as cancer and other diseases [15, 16]. This small nanovesicle, which was first discovered in the 1980s, was defined as cellular waste resulting from cell damage, or by-products of cell homeostasis [17]. But with recent studies, it was seen that this structure has many features such as the regulation of immune system response, intercellular communication, signal transmission, and genetic material transfer [18-20]. The structure takes shape with the inward budding of the multivesicular body (MVB) membrane and releases by practically all eukaryotic cells [21]. It is an average diameter of 30-150 nm and has membrane and cytosolic components that include proteins, lipids, DNA, and RNAs [22-25]. Undoubtedly, being nano-sized and containing nucleic acid adds a natural carrier feature to exosomes. In particular, their biological distributions and high plasma stabilities offer the possibility of use for tumor therapeutic applications.

The first therapeutic avenue is to use exosomes as an exogenous molecule carrier. In a study conducted in 2019, Wang et al. demonstrated that macrophage-derived exosomes that loaded with paclitaxel display strong anti-tumor activity [26].

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In another study, Tomasetti et al. reported that intestinal-type sinonasal adenocarcinomas-derived exosomes that loaded with miR-126 inhibited cell growth and the tumorigenic potential of sinonasal cancer cells [27]. In this way, anti-cancer drugs, miRNAs, and functional RNAs are loaded into exosomes using highly efficient exosomal loading methods and carried out the treatment.

Another therapeutic avenue is to use exosomes derived from tumors or other cells directly. Tumor-originated exosomes have many roles that affect tumor growth and development. These exosomes produce immune responses against tumor cells as they have the molecules needed for antigen presentation such as MHC/peptide complex [28]. In this context, the same year, Escudier et al. and Morse et al. performed a phase I clinical study in dendritic cell-derived exosomes (Dex) [29, 30]. Evidence has revealed that Dex may stimulate tumor cell-specific cytolysis and having the potential to produce immune responses against tumor cells. Similarly, Dai et al. reported that autologous acid-derived exosomes (Aex) obtained from colorectal cancer patients induced tumor immunity effectively [31]. No doubt, exosomes represent a promising solution in nanotechnology with their high drug loading capacity and specificity, low immunogenicity characteristic, and non-cytotoxic impact. Nevertheless, the clinical implementations of exosomes are still in the beginning stage due to their isolation and characterization problems. Though lately established technologies can decrease these problems, additional challenges include inadequate quality control and standardization across the study groups. That is why we need to understand the exosome formation and the mechanism in depth.

In this review, we have examined under two main headings the use of exosomes in cancer treatment: (a) use of exosomes on their own and (b) use of exosomes as an exogenous molecule carrier. Additionally, in the review, exosome characterization and composition, biogenesis and functions, advantages and disadvantages are summarized. As a result, we provide an overview of exosome studies with an emphasis on current developments in exosome-mediated cancer targeting therapy.

An overview of the exosome biology

The endosomal pathway is completed by two basic formations classified as early and late [32]. Exosomes occur within this pathway during the maturation of endosomes. While the early endosome matures towards the late endosome, the endosome membrane makes a series of inward recesses and forms many exosomes/intraluminal vesicles (ILVs) structures [21]. These maturing late endosomes have been defined as multivesicular endosomes (MVEs) or multivesicular bodies (MVBs) by Sotelo and Porter in the 1959s [33]. Then, MVBs/MVEs fuse with the plasma membrane, and exosomes release into the extracellular environment [34,35]. During this process, many components are the endosomal sorting complexes required for transport (ESCRT), tetraspanins, signaling transducing adaptor molecule (STAM1), Alg-2 interacting protein X (Alix), Hrs, various lipid-modifying enzymes, tumor susceptibility gene 101 (Tsg101), etc., that involve in the formation of MVEs and ILVs [36-39].

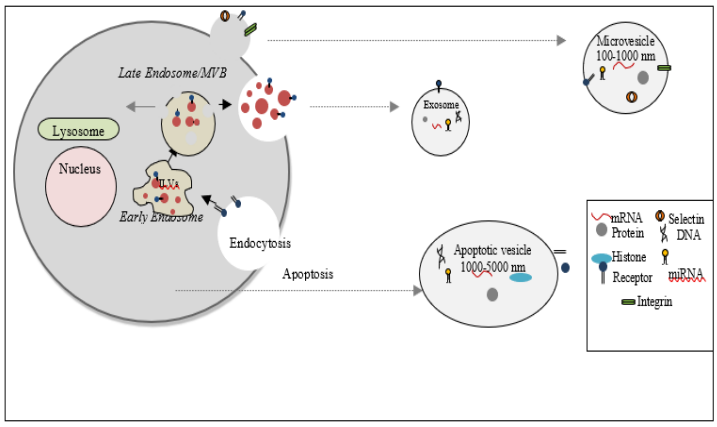

Fig 1. Exosome biosynthesis and other extracellular vesicles forms

(a) exosome biogenesis, (b) transport of MVBs to the plasma membrane, and (c) fusion of MVBs with the plasma membrane. In the endocytic pathway, extracellular vesicles (EVs) are released such as apoptotic bodies, microvesicles (MVs), and exosomes. MVs are 100-1000 nm in size and have a heterogeneous population. Apoptotic bodies are 1000-5000 nm in size and are closed structures with higher sucrose gradient density than MVs [40]. However, exosomes have a heterogeneous population in contrast to the known exosome definition: large-exosome (90-120 nm), small-exosome (60-80 nm), and non-membrane exomer (<50 nm) [41, 42]

According to their endosomal origin, exosomes from different cell types contain proteins involved in membrane transport and fusion, MVEs biogenesis, and adhesion proteins such as integrins and specific tetraspanins. Nevertheless, heat shock proteins and cytoskeletal proteins are part of the "signature" of exosomes [22-24]. Exosomes contain not only these proteins but also many mRNAs that can incorporate into recipient cells. Studies have shown that there are a lot of types of RNA are present in exosomes, including microRNA (miRNA), messenger RNA (mRNA), ribosomal RNA (rRNA), Y-RNA, vault RNA, long non-coding RNA (lncRNA), circular RNA (circRNA) and transfer RNA (tRNA) [43-47]. In particular, there are many exosomal miRNAs which are used as prognostic markers (e.g., miR-224 in hepatocellular carcinoma [48], miR-301a and miR-23a in colorectal cancer [49], miR-375 and miR-1307 in ovarian cancer [50]. Also, some exosomal miRNAs (e.g., miR-214, miR-29a, miR-1, miR-126, and miR-320) have been reported to participate in exocytosis, angiogenesis, hematopoiesis, and tumorigenesis [51]. However, recent developments have shown that exosomes are rich in lipids such as cholesterol, sphingomyelin, etc. No doubt, the lipid and DNA content of exosomes, like protein and RNA biomarkers can serve as the molecular signature for disease diagnosis and prognosis.

With the clinical use of exosomes, it has become imperative to optimize their isolation method for maximum yield, purity, and test reproducibility. Interestingly, although exosomes found in almost all body fluids including cerebrospinal fluid [52], blood, urine [53], semen [54], amniotic fluid [55], saliva [56], and breast milk [57], high purity exosome isolation is not yet available. For instance, though differential ultracentrifugation is currently considered as the gold standard of exosome isolation, exosomes isolated by using this method often contain proteins and lipoproteins. Likewise, ultrafiltration can suffer from clogging and vesicle trapping even though it is a rather popular exosome isolation technique [58]. Also, exosomes are isolated by using immune affinity capture [59], size exclusion chromatography [60], commercially available kits, or microfluidic technologies. But, techniques inside this group also bring about a unique set of disadvantages and advantages to exosome isolation.

Exosome-mediated cancer treatment

Chemotherapy, radiotherapy, and immunotherapy methods are used as conventional treatment methods in cancer treatment. But, off-target effects and treatment resistance are significant problems for these methods. Thus, effective drug delivery systems that can deliver drugs specifically to the tumor will positively affect the treatment results. In recent years, several studies have shown that exosomes can be used to treat many types of cancer [29-31].

Use of exosomes on their own

The molecular composition of exosomes generally reflects the parental-cell-type specificity. Notably, cancer-derived exosomes include cancer-specific antigens expressed in the parental cancer cells. Studies have shown that in most cancer-derived exosomes have cancer antigens such as Melan-A [61], Silv [62], carcinoembryonic antigen [63], and mesothelin [64]. Except for the common antigens, cancer-derived exosomes also contain the MHC/peptide complex that reflects their

Table 1. Common molecular content of exosomes

Exosome Content	Examples
Proteins	Tetraspanin: CD9, CD63, CD81, CD82 Heat shock proteins: hsp60, hsp70, hsp90 Membran transport and fusion proteins: GTPase, annexin 1-2 Alix, Tsg101 Signal proteins: EGFR, CDC42, ARF-1, β-Catenin *etc.*, Cytoskeletal proteins: Actin, tubulin, myocin *etc.*,
Lipids	Phosphatidylserine (PS) Cholesterol Sphingomyelin (SM)
Nucleic acids	mRNA miRNA mitochondrial DNA (mtDNA) chromosomal DNA

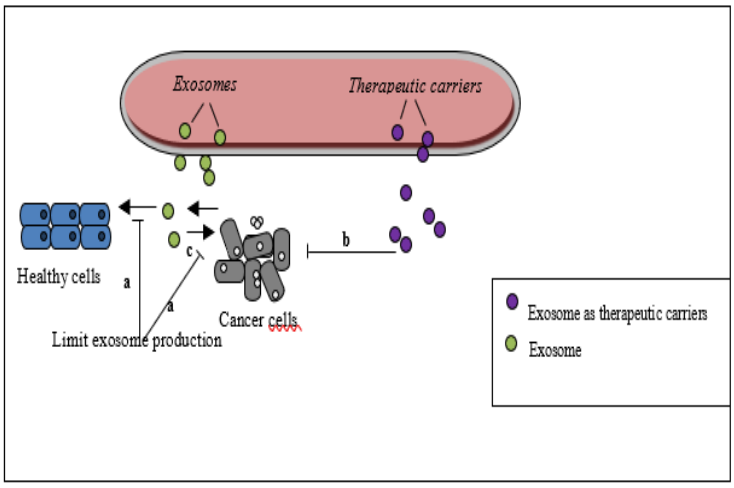

Fig 2. Exosome based cancer therapeutic strategies
(a) remove specific exosomes or prevent exosome production for suppresses tumor progression, (b) use of exosomes as an exogenous molecule carrier, and (c) use of exosomes on their own

cellular origins. Exosomes derived from these antigen-presenting cells (APCs) play an important role in the regulation of the antitumor immune system [65-67]. Undoubtedly, this suggests exosome-based cancer vaccines can be developed by releasing antigens by APCs. Indeed, dendritic cells, the antigen-presenting cells of the immune system have been used in the immunotherapy method due to their potential to activate T cells. Wolfers et al., have shown in the *in vitro* model system, exosomes secreted by tumor cells include tumor antigens and have dendritic cells. In the study it was observed that induced potent CD8+T cell-dependent antitumor effects on mouse tumors of dendritic cells derived from exosomes (Dex) [62]. In another study, Escudier et al., reported the applicability of the Phase I experiment using the autologous Dex for the immunization of stage III/ IV melanoma patients. Results have revealed that Dex is capable of supporting tumor cell-specific cytolysis and eradicating the growth of murine tumors [29]. A similar study has performed using the second generation of Dex, exosomes derived from interferon (IFN)-γ-maturated dendritic cells (IFN-γ-Dex) pulsed with tumor-associated antigenic peptides. In the study, IFN-γ-Dex has seen to increase the natural killer cell functions and related antitumor immunity in the NKp30-dependent manner [68]. With the studies carried out in recent years, to further improve the functionality and effectiveness of Dex, engineered Dex has been developed. The main target of the study is to transport proteins or mRNAs associated with the tumor-antigen via Dex [69,70]. Although Dex has a great potential to generate immune responses against tumors, the clinical progression of this application is still in its early stages. Applying this kind of therapy in large populations is costly and needs the monitoring of well-defined quality control parameters [71]. Besides Dex, ascites-derived exosomes (Aex) are possible to be used as a cell-free tumor vaccine in the immunotherapy of cancer. In phase I clinical study conducted in 2008, it was seen that a positive adjuvant in the induction of antitumor immune responses combined with granulocyte-macrophage colony-stimulating factor (GM-CSF) of Aex obtained from colorectal cancer patients [31]. As a new vaccine strategy for cancer immunotherapy, Aex will considerably improve the clinical outcomes with the exact cellular origin of exosomes and further characterization. However, donor cell type and sending method are also a crucial issue for cancer treatment. We should never forget the fact that tumor cells may use exosome secretion as a way to survive under stress [72,73].

Undoubtedly, since exosomes positively affect tumor progression and metastasis the prevention of exosomal release is another treatment method. In 2010, Kosaka et al., aimed to decrease the formation of exosomes using an inhibitor that prevents the formation of ceramide [74]. In another study Chalmin et al., since exosome release has induced by an increase in the amount of intracellular Ca^{+2}, it targeted the reduction of exosome formation as a result of amiloride suppression of Ca^{+2} channels [75].

Table 2. Exosome-mediated cancer vaccine studies

Cargo	Exosome source	Cancer type	Phase	References
Tumor antigenic peptides	Dendritic cell-derived	Melanoma	I	[29]
Tumor antigenic peptides	Dendritic cell-derived	Non-small lung cancer	I	[30]
Tumor antigenic peptides	Ascites-derived	Ovarian cancer	-	[76]
-	Ascites-derived	Colon cancer	I	[31]
Tumor antigenic peptides	Renal cancer-derived	Renal cancer	*in vivo*	[77]
Tumor antigenic peptides	Dendritic cell-derived	Malignant glioma	*ex vivo*	[78]
Chemokine	Heat-stressed tumor cells	Lung carcinoma	-	[79]
Tumor antigenic peptides	Dendritic cell-derived	Mouse tumor	-	[80]
Tumor antigenic peptides	IFN-γ-matured dendritic cells pulsed with antigenic peptides	Advanced lung cancer	II	[68]

Use of exosomes as a molecule carrier

There have been considerable advances in drug design and delivery thanks to the development of nanotechnology in the 21st century. With these developments; there have been designed several drug delivery vehicles such as polymeric micelles, carbon-based and lipoprotein-based drug carriers, liposomes, and dendrimers [6]. Liposomes and polymeric nanoparticles are the most preferred drug delivery vehicles. However, biocompatibility and long-term potential safety of these structures remain a concern [81]. In these circumstances, exosomes or exosome mimetics [82] appear to be a superior choice that overcomes these concerns [83]. Especially, their long-circulating half-life, the intrinsic ability to target tissues, biocompatibility, and minimal or no inherent toxicity issues [84] offer the possibility of use for tumor treatment. Commonly, short interfering-RNA (siRNA), microRNA (miRNA), recombinant proteins, and especially anti-cancer drugs can be encapsulated for exosome-mediated administration by various delivery techniques [85]. In this way, they can provide a powerful biological effect on the target cells. These encapsulated exosomes then are capable of delivering their cargos across the blood-brain barrier and confer a powerful biological effect on target cells [13,14]. In one of the first reports, a group in Oxford showed for the first time that siRNA-loaded exosomes had therapeutic potential by a knockdown of the levels of BACE1 (a therapeutic target in Alzheimer's disease) in the brain. In the study, exosomes have transferred siRNA specifically to neurons, microglia, and oligodendrocytes in the brain, resulting in the BACE1 gene knockdown [86]. However, exosomes can also be chemically or biologically modified to yield delivery systems that can improve the treatment results of chemotherapeutic drugs, as well as decrease drug toxicity. In 2019, Yu et al. demonstrated that human fetal lung fibroblast-originate exosomes loaded with erastin chemo drug suppressed MDA-MB-231 cell proliferation and migration [87]. In another study, Pascucci et al., observed that paclitaxel-loaded mesenchymal stromal cells (MSCs) induced cell proliferation and tumor growth by 50% in a dose-dependent manner [88]. Finally, in a study on Celastrol (CEL) demonstrated that exosomes loaded with CEL exhibited enhanced anti-tumor efficacy as compared to free CEL against lung cancer cell xenograft [89].

Advantages and disadvantages of exosome-mediated cancer treatment

Tumor therapeutic use of exosomes is an exciting and quickly evolving field of research, with great potential in the healing of cancer patients. However, there remain considerable challenges to overcome including the co-isolation of the potential damage of exosomes, non-exosomal impurities, low RNA yield, and low-throughput of samples. Considering all these disadvantages, as the role of exosomes in cancer progression become more apparent, there will be increased efforts towards their clinical application too [107].

Table 3. Exosome-mediated drug delivery system studies in cancer treatment

Cargo	Exosome source	Cancer Type	Results	References
miR-146b	marrow stromal cell	primary brain tumor	reduced in glioma xenograft growth	[90]
Let-7a	exosomes with the GE11 peptide on the surface	breast cancer	therapeutic target	[91]
Doxorubicin	mouse immature dendritic cells	positive breast cancer	therapeutic target	[92]
Paclitaxel	mesenchymal stromal cells	CFPAC-1 pancreatic cell line	a strong anti-proliferative activity	[88]
miR-122	AMSC	hepatocellular carcinoma	increased anti-tumor activity *in vivo*	[93]
miR-134	Hs578T and Hs578Ts(i)$_8$ cells	triple-negative breast cancer	reduces triple-negative breast cancer aggression and increases drug sensitivity	[94]
Doxorubicin	breast cancer cell	breast and ovarian cancer	increases the therapeutic index	[95]
Doxorubicin	blood	murin hepatoma	enhanced cancer targeting under an external magnetic field and suppressed tumor growth	[96]
Paclitaxel	macrophage	multiple drug resistance cancer cells	therapeutic target	[97]
Celastrol	bovine milk	lung cancer	improves therapeutic potential	[89]
miR-21	tumor-associated macrophages	gastric cancer	increased cisplatin drug resistance	[98]
UCH-L1	MCF7/ADM	MCF7/WT	significantly negatively correlated with prognosis	[99]
miR-126	intestinal-type sinonasal adenocarcinomas	malignant nasal-septum carcinomas	inhibited cell growth and the tumorigenic potential of cells	[27]
miR-27a	gastric cancer	cancer-associated fibroblasts	a potential therapeutic target in the treatment	[100]
TRIM3	gastric cancer	gastric cancer	suppress gastric cancer growth and metastasis *in vitro* and *in vivo*	[101]
Paclitaxel	pro-inflammatory M1-macrophage cells	MDA-MB-231, MCF-7, 4T1, A549, HepG$_2$ and Hela cells	the anti-tumor effects of paclitaxel was significantly improved	[26]
lncRNA AGAP2-AS1	breast cancers	SKBR-3 and BT474 breast cell line	AGAP2-AS1 promotes trastuzumab resistance of breast cancer cells	[102]
Erastin	HFL-1	triple-negative breast cancer	suppressed breast cancer cell proliferation and migration	[87]

AMSC: adipose tissue-derived mesenchymal stem cells AGAP2-AS1: AGAP2 antisense RNA-1 HFL-1: human fetal lung fibroblasts MCF7/ADM: adriamycin-resistant human breast cancer cells MCF7/WT: adriamycin-sensitive human breast cancer cells TRIM3: Tripartite motif-containing protein 3 UCH-L1: ubiquitin carboxy-terminal hydrolase L1

Table 4. Advantages and disadvantages exosomes in cancer treatment

Advantages	Disadvantages
Low toxicity and immunogenicity [103]	Effects on target organs and the therapeutic effect is not yet clear.
Easily cleared from the lung and passes easily through the blood brain barrier [104,105]	High purity exosome isolation is not yet available.
Binding and expression to tumor cells is higher than liposomes of the same size [106]	Caspase-3 carrying of exosomes may prevent cell death by apoptosis and may cause tumor cell survival.
It is easily produced by cells.	Clinical use should be reliably characterized.

CONCLUSION

As a result, exosomes have important biological and morphological properties, making it possible to use these nanovesicles in both treatment-oriented and diagnostic applications. However, some important problems with the use of exosomes continue. There are some obstacles such as high purity not obtained and the lack of a clear therapeutic effect in clinical practice. A better understanding of exosome biology will help to overcome these problems and develop new therapeutic approaches. At the same time, with the development of techniques to provide more sensitive miRNA detection, the potential power of exosomal miRNAs in the diagnosis of the disease will be increased and the clinic will be used more effectively and widely. In addition, the complex structure of exosomes can limit their pharmaceutical acceptability. The various components of exosomes may be incorporated into liposomes or nanoparticles to enhance stability, immunogenicity, targeting delivery, and uptake.

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