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EXPERT REVIEW

Exosomes as Carriers for Drug Delivery in Cancer Therapy

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Abstract
Exosomes are extracellular vesicles secreted by cells with a particle size of 30–150 nm in diameter. Exosomes can be used as natural drug carriers. The treatment of cancer with drug-loaded exosomes is an area of high interest. This review introduces the composition, function, isolation and characterization of exosomes, and briefly describes the selection of exosome donor cells and methods for drug loading. Through studies on therapies with drug-loaded exosomes in gastric cancer, lung cancer, brain cancer and other cancers, the advantages and disadvantages of drug-loaded exosomes have been analyzed.

Key Words Cancer therapy · Drug delivery · Extracellular vesicles · Exosomes

Introduction

Cancer has become a research hotspot because of its high mortality and incurability. Common treatments for cancer therapy include surgery, chemotherapy, radiotherapy, immunotherapy, small chemical molecules and small biological molecules (including RNA and exosomes). Exosomes are a subpopulation of extracellular vesicles secreted by cells with a particle size of 30–150 nm in diameter that exist in various body fluids[1, 2]. Exosomes was first discovered in reticulocytes in 1983[3] and named in 1987[4]. Exosome release was originally thought to be a way for cells to excrete waste. Until 1996, Graca Raposo *et al*. found that exosomes released from B lymphoblastoid cells had antitumor effect[5]. Exosomes contain lipids, proteins, nucleic acids and other substances. In 2007, Hadi Valadi *et al*. found that mRNA and microRNA in exosomes could be transferred between cells, resulting in genetic exchange[6]. In the last decade, researches on the exosome field has made advanced progress rapidly. A large number of studies on exosomes as liquid biopsy and drug delivery platforms have been carried out. Due to the overlap of size and shape between exosomes and small-sized macrovesicles (with size less than 200 nm in diameter), it is difficult to obtain relatively pure preparation and characterize properly, leading to controversy over the term of exosome. In 2018, the International Society for Extracellular Vesicles proposed using small extracellular vesicles (sEVs) instead of exosomes as an optional term for EVs with a size less than 200 nm in diameter[7]. However, in the last decade, the term of exosome still has been widely used in scientific publications. For the convenience of readers with different expertise, we will keep using the word of exosome in this review.

The formation of exosomes mainly includes the following steps: firstly, endocytic vesicles are formed through plasmalemma invagination; secondly, early endosomes form and then mature into late endosomes; thirdly, with the entry of "cargo", such as cytoplasmic nucleic acids and proteins, many intraluminal vesicles (ILVs) are produced in

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late endosomes, and endosomes are then transformed into multivesicular bodies (MVBs) through inward budding; and finally, MVBs can fuse with lysosomes, which can degrade ILVs. On the other hand, MVBs can be transported to and then fuse with the plasma membrane to secrete ILVs outside the cell[4, 8, 9]. In this scenario, the ILVs are considered exosomes.

Exosomes derived from different cells have different functions, such as affecting tumor invasion[10], tissue repair[11], and immune regulation[12] and using in drug delivery [13, 14]. At present, drug carriers consist of nanoparticles, liposomes, natural or synthetic macromolecular materials, etc. Drug carriers can control the rate of drug release and actively or passively deliver drugs to the target site, avoiding the limitations of general drug therapy and being beneficial to the treatment of tumors; however, these drug carriers also have some drawbacks. As foreign substances, nanoparticles can induce an immune response, which is characterized by poor biocompatibility. In addition, they can be cleared by the mononuclear phagocyte system, leading to reduced efficacy. In contrast, exosomes, vesicles secreted by the body's own cells, have good biocompatibility and do not cause immune reactions[15]. Elimination by the mononuclear phagocyte system can be avoided, which leads to a long cyclic half-life. The plasma half-life of BAY55-9837 loaded in exosome-SPION was reported to be 27-fold longer than that of BAY55-9837 alone[16]. Therefore, exosomes are a promising carrier for drug delivery. Studies in this field are of great significance for cancer treatment. This review summarizes the basic characteristics of exosomes, the application of exosomes as carriers for cancer treatment (in gastric cancer, lung cancer, brain cancer and other cancers), the selection of cell sources of exosomes, drug-loading methods, and the advantages and disadvantages of exosomes as drug carriers.

Isolation and characterization of exosomes

To load drugs and better study the composition and functions of exosomes, it is necessary to separate exosomes from cell culture medium, biological fluids or tissues. At present, there are various methods for exosome isolation, but there is no gold standard. The commonly used methods for the isolation of exosomes include ultracentrifugation (UC), ultrafiltration, immunoaffinity capture methods, chromatography, etc.

One of the most common isolation techniques is UC. UC can be divided into differential UC and density gradient UC[17]. The procedure for differential ultracentrifugation is to first remove dead cells and cell fragments by low-speed centrifugation and then to precipitate exosomes by ultracentrifugation steps (with a centrifugal force of 100,000–120,000 × g) according to the difference in sizes among the substances. This results in a mixture of exosomes and some contaminating proteins, so the precipitate needs to be resuspended in PBS and centrifuged again. Finally, the supernatant is removed to obtain purer exosomes. It should be noted that centrifugation operations should always be conducted at 4 °C[18]. The theory of density gradient centrifugation is that when there is a difference in the sedimentation coefficient between different particles, under a certain centrifugal force, the particles with each coefficient settle at a certain speed, ultimately forming zones in different areas of the density gradient. The commonly used density gradient solvents are sucrose and iodixanol[19]. Both sucrose and iodixanol are inert solutions capable of forming density gradient solutions. Sucrose is readily soluble in water. Exosomes could float in a sucrose gradient solution with a density of 1.10–1.18 g/ml, which is close to the density of exosomes (1.13–1.19 g/ml)[20]. Iodixanol is a highly hydrophilic substance that can form a continuous or discontinuous density gradient solution. Iodixanol has some advantages over sucrose. Firstly, it can form iso-osmotic solutions at all densities, which helps preserve the size and shape of isolated exosomes[21]; Secondly, iodixanol density gradients could accurately separate different subtypes of EVs, while the sucrose density gradients could not[22]; and thirdly, iodixanol density gradients enables the separation of other substances which has similar densities to exosomes, such as viruses and microparticles. But sucrose density gradients cannot effectively separate them[23].

Ultrafiltration can use ultrafiltration membranes with a certain size pore or molecular weight cutoff (MWCO) to selectively separate exosomes under the action of centrifugal force. Zhang *et al*. [24] used a 0.22-μm filter to isolate exosomes derived from human umbilical cord mesenchymal stem cells (MSCs). The ultrafiltration method is simple to perform and has a low cost. Additionally, He *et al*. [25] optimized an ultrafiltration method by passing urine samples sequentially through a 0.22-μm filter and a dialysis membrane with an MWCO of 10,000 kDa. Their results demonstrated that this optimized method could obtain exosomes with higher purity.

The immunoaffinity capture approach is also called the immunomagnetic bead method. There are many specific markers in the exosome membrane, such as CD9, CD63, CD81, TSG101, and Alix[26, 27]. By incubating exosomes with magnetic beads coated with specific antibodies, antigens can bind to the antibodies on the magnetic beads through antigen–antibody interactions[28]. Thus, exosomes can be specifically captured. In addition to the exosomal markers mentioned above, exosomes from certain cells may also express specific markers. For example, exosomes derived from melanoma cells express chondroitin sulfate peptidoglycan 4(CSPG4), whereas normal cell-derived

exosomes do not. Therefore, exosomes from melanoma cells can be captured using anti-CSPG4 monoclonal antibodies (mAbs) by the immunoaffinity capture method[29].

In addition to the commonly used methods described above, exosome isolation methods also include size exclusion chromatography (SEC)[30], a polyethylene glycol (PEG)-based precipitation method[31], an exosome isolation kit method[32], and microfluidics[33]. These techniques for isolating exosomes have their own advantages and limitations, and the purity, recovery rate, and stability of the isolated exosomes vary (Table I). When UC and SEC were compared for exosome isolations, it was found that fewer exosomes were recovered by the UC, but the purity was higher than that achieved with SEC[34]. Compared with SEC, combined use of UC and SEC can obtain better separation results[35]. In the separation of exosomes released from human colon cancer cells, Tauro *et al*. compared UC, density gradient centrifugation and immunoaffinity capture methods and found that the immunoaffinity capture method was the best[36]. The appropriate isolation method can be selected according to downstream analyses and applications. Sometimes two or more isolation methods can be used in combination to exploit their advantages and make up for their defects. A study showed that the number of exosomes separated by combined use of ultrafiltration and SEC could be up to 58 times than that isolated with UC[37].

After separation and purification, isolated substances need to be characterized to verify whether they are exosomes. The characterization of exosomes is based on the size, morphology, density, and protein content of exosomes. Commonly used exosomal characterization methods include electron microscopy (including scanning electron microscopy and transmission electron microscopy (TEM)), western blot analysis, enzyme-linked immunosorbent assay, FACS analysis of labeled exosomes bound to beads, dynamic light scattering (DLS) and nanoparticle tracking analysis (NTA) [38–40]. The size of exosomes can be determined by DLS and NTA, and many studies have reported that the size of exosomes is 30–150 nm[41, 42]. TEM can be used to observe the morphology of exosomes. Zhu *et al*. found that macrophage-derived exosomes and nicotine-induced macrophage-derived exosomes are cup or sphere shaped[43]. In addition, the proteins enriched in exosomes and proteins deficient in exosomes can be used together to characterize exosomes. Jan Lötvall *et al*. [44] found that the proteins enriched in exosomes include tetraspanins (CD9, CD63, and CD81), integrins, and endosome- or membrane-binding proteins (TSG101, annexins, and Rabs). The proteins lacking in exosomes are HSP90B1, GM1330, CYC1, etc.

Composition and functions of exosomes

The main components of exosomes include proteins (such as tetraspanins, enzymes, transcription factors, and heat shock proteins), lipids, and RNAs (such as mRNA, miRNA, lncRNA and cirRNA) (Fig. 1)[54–57]. There are several exosome-related databases, such as ExoCarta[58], Vesiclepedia[59], and exoRBase[60]. These databases can provide information about proteins, lipids and RNAs in exosomes. However, some contents of exosomes are present in only exosomes derived from special cells, and some contents are common among all exosomes. Obviously, the content of exosomes derived from different cells varies; for example, the proteins and lipids contained in exosomes isolated from ovarian surface epithelial cells and ovarian cancer cells are clearly different[61]. The physiological and pathological states of cells also have an impact on the composition of exosomes. One study found that the level of the lncRNA PTENP1 was lower in the plasma exosomes of bladder cancer patients than in those of healthy controls[62].

Table I Comparison of Common Methods for Exosome Isolation

Methods	Principle	Benefits	Limitations	References
Differential ultracentrifugation (DU)	Based on size	Low cost, High yield	Protein contamination, Poor integrity of exosomes	[45, 46]
Density gradient ultracentrifugation	Based on density	Higher recovery rate than DU, Higher purity than DU	Expensive, High time consumption	[21, 47]
Ultrafiltration	Based on size	High exosomes yields, Time saving	Poor exosomal integrity	[48, 49]
Size exclusion chromatography	Based on size	Less albumin contamination, Higher purity than DU	Low yield, sample volume limitation	[50, 51]
Immunoaffinity capture	Based on antigen–antibody binding	High recovery rate, high yield	Limitation of antibody availability and suitability of exosome markers	[36, 52]
PEG-base precipitation	Based on solubility (solvent precipitation)	High recovery rate, Low cost	Low purity	[31, 53]

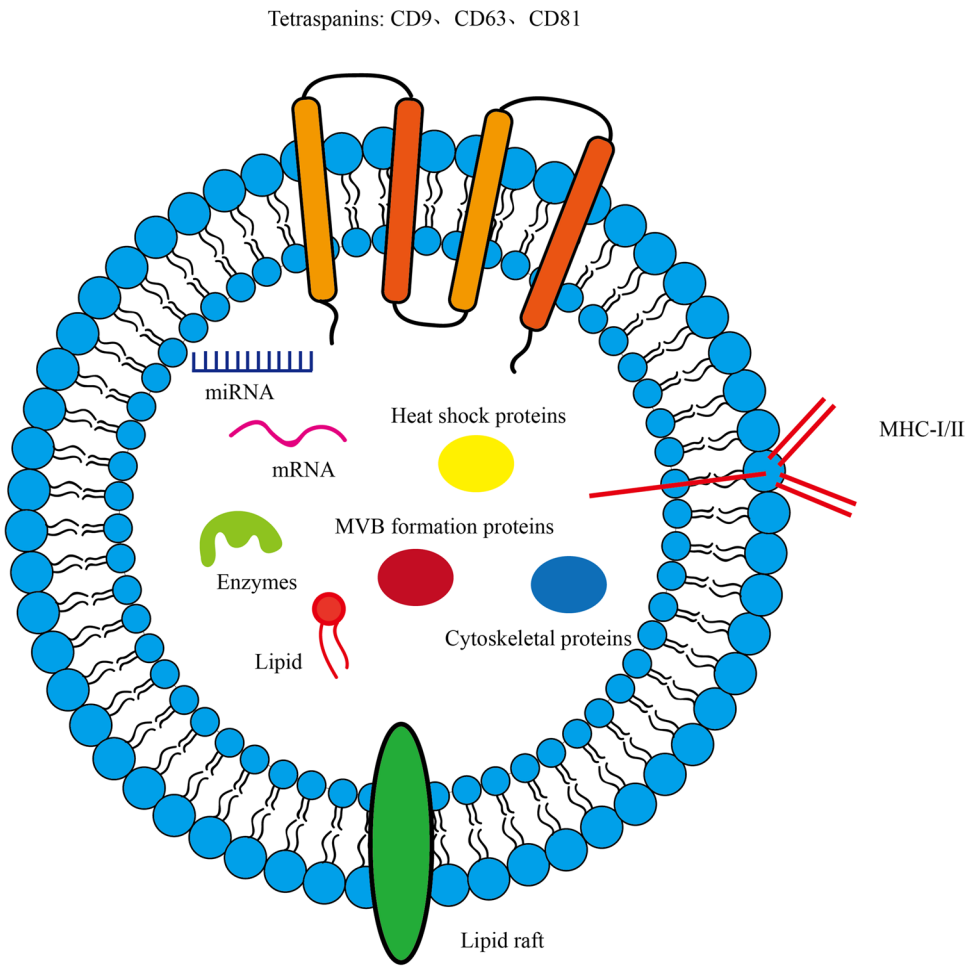

Fig. 1 The Main Components of Exosomes

At present, there are some controversies about the inclusion of DNA in exosomes. Many studies have shown the presence of DNA in exosomes[63, 64]. Moreover, Takahashi *et al*. [65] stated the opinion that exosomes derived from some cells can maintain cellular homeostasis by releasing harmful cytoplasmic DNA outside the cell. In contrast, Jeppesen *et al*. [66] used high-resolution density gradient centrifugation combined with direct immunoaffinity capture to isolate exosomes, and found that double-stranded DNA and DNA-binding histones were not detected in exosomes by nucleic acid analysis. The difference may be because different studies use exosomes produced by different cells, or because of the different isolation methods, the existence of other extracellular vesicles or non-vesicular substances may ultimately affect the experimental results. The composition of exosomes has not been fully determined; thus, more in-depth research is needed.

The formation of exosomes was described in the introduction. In the process of exosome generation, there are some mechanisms that affect the sorting of endogenous cargoes into exosomes. Cargo sorting mechanisms can be divided into two types: the endosomal sorting complex required for transport (ESCRT)-dependent mechanism and ESCRT-independent mechanisms. The ESCRT-dependent mechanism plays a key role. The ESCRT-dependent mechanism involves the ESCRT-0, ESCRT-I, ESCRT-II and ESCRT-III pathways. They work together to recognize and capture ubiquitinated proteins, and these proteins can enter endosomes through invagination and rupture of the endosomal membrane[67]. ESCRT-independent mechanisms include lipid raft- and ceramide-mediated mechanisms, which also modulate the composition of exosomes[68].

The biological functions of exosomes have been gradually elucidated with increasing research on exosomes. Exosomes are known to be involved in intercellular communication, disease diagnosis and treatment, tissue repair, and drug delivery. However, different types of cells produce exosomes with different functions; for example, exosomes from cardiac progenitor cells can inhibit cardiomyocyte apoptosis, thereby protecting the heart[69], while gastric cancer cell-derived exosomes can promote the proliferation and metastasis of gastric cancer cells by inducing neutrophil tumor-promoting activity[70]. There are also some studies showing that exosomes can be used as carriers for drug delivery; for

example, exosomes derived from HEK293T cells and loaded with the superrepressor IκB can reduce the mortality of septic mice[71]. In this review, the role of exosomes as drug carriers is the main topic discussed below.

Selection of donor cells for exosomes

Almost all cells release exosomes, and exosomes are present in all body fluids[72]. Exosomes derived from different types of cells are different. Their drug-loading efficiency, yield and antitumor capacity vary. Kanchanapally *et al*. found that pancreatic cancer cells released more exosomes than pancreatic stellate cells and macrophages and that the released exosomes had the highest loading efficiency for doxorubicin. However, doxorubicin-loaded and macrophage-derived exosomes had the strongest antitumor effects[73]. It was also found that the inhibitory effects of taxol-loaded exosomes from different cell sources on A549 lung cancer cells exhibited the following hierarchies: taxol-loaded exosomes secreted by HuVEC-4 cells < taxol-loaded exosomes secreted by HuVEC-6 cells < taxol-loaded exosomes secreted by MSCs[74]. These results may be because the contents of exosomes are partially derived from the parental cells[75], which also plays a role in cancer treatment to some extent. Therefore, the efficacy of drug-loaded exosomes secreted by different cell types will be different. In addition, a study found that the distribution of exosomes *in vivo* could be influenced by the cell source of exosomes and the route of administration. HEK293T cell-derived exosomes were mainly distributed in the liver, spleen and gastrointestinal tract after intravenous injection[76]. Exosomes from some cell types have natural targeting capabilities; for example, exosomes from endothelial cells are more likely to be distributed in the bone than those secreted by osteoblasts or bone marrow MSCs[77]. For cancer treatment, in addition to exploiting the natural targeting capabilities of exosomes, exosomes can be engineered to accumulate at the cancer site[78].

Methods of loading exosomes with drugs

As natural drug delivery vehicles, exosomes can be loaded with chemotherapeutic drugs, therapeutic nucleic acids, proteins, etc. It has been reported that there are many approaches for loading drugs into exosomes for the purpose of treating many diseases (Table II). We speculate that the selection of exosome donor cells, the types of drugs, and the methods of drug loading would all affect the efficacy of exosome-based drug delivery systems. Here, we divide the methods for loading drugs into exosomes into two categories: 1) treatment of cells with drugs before the isolation of exosomes from the cells, so that the cells can release drug-containing exosomes and 2) loading of drugs into exosomes after isolation. An overview of these methods is provided below.

Treating cells before exosome isolation

Drug loading can be performed before exosomes are released from cells by transfection or mixing cells with drugs. Transfection methods include liposomal transfection, electroporation transfection, and viral infection. Hepatocyte growth factor (HGF)-specific siRNA can be transferred into HEK293T cells by using a transfection reagent; thus, the transfected cells will produce exosomes encapsulating HGF-specific siRNA. These exosomes are then taken up by gastric cancer cells, in which they play a role in inhibiting tumor growth[79]. Kalimuthu *et al*. found that mixing MSCs with paclitaxel (PTX) and then extruding the cells using an

Table II Different Drug Loading Methods of Exosomes and the Applications

Source of exosomes	Cargo	Loading methods	Application	Reference
Raw 264.7 macrophage	catalase	the incubation at RT with or without saponin freeze–thaw cycles Sonication Extrusion	Parkinson's Disease	[13]
Human Embryonic Kidney 293 T cells (HEK293T)	HGF siRNA	transfection into cells	Gastric Cancer	[79]
human peripheral blood	miRNA (miR-21 mimic or inhibitor)	transfection into exosomes	cardiac diseases	[80]
HEK293T cells	miRNA-497	transfection into exosomes	Non-small cell lung cancer (NSCLC)	[81]
glioblastoma U87 cells	$hsiRNA^{HTT}$	co-incubation	Huntington's disease	[82]
transduced MSCs	doxorubicin (DOX)	electroporation	targeted cancer therapy	[83]

extruder with a polycarbonate membrane could obtain PTX-loaded exosome mimics[84].

Although there are many studies on loading chemotherapeutic drugs and RNA, it is difficult to load proteins into exosomes by the commonly used methods described above [85]. To solve this problem, some new approaches for loading proteins into exosomes have emerged. These methods are linked to the biogenesis process of exosomes. Yim *et al*. [86] designed "exosomes for protein loading via optically reversible protein–protein interactions" (EXPLORs) for protein delivery; this technology forms protein–protein modules by using two proteins, CIBN and CRY2. Under blue light, CIBN and CRY2 combine together, so CIBN-conjugated CD9 (an exosomal membrane protein) and a CRY2-conjugated target protein would bind, allowing the target protein to enter exosomes. After turning off the light source, the target protein can be separated from CD9 on the membrane and enter the exosomal cavity so that the target protein can enter target cells. In addition, another study showed that the late-domain-containing protein Ndfip1 could recognize a WW-tagged protein, driving ubiquitination and exosomal entry of the tagged protein during the exosomal biological process[87].

Loading drugs after exosome isolation

Methods for loading drugs after exosome isolation include electroporation, coincubation of exosomes with drugs at room temperature, sonication, extrusion, dialysis, and freeze–thaw cycling. The method of co-incubating exosomes with drugs is simple. Incubating exosomes derived from raw bovine milk with curcumin at room temperature can achieve a drug loading rate of 18–24%[88]. Sometimes saponin is added to assist in loading, that is, exosomes are incubated with drugs and saponins at room temperature[89]. When electroporation is used to load drugs, the electric current breaks through the exosomal membrane reversely, and temporary pores form in the lipid bilayer of the exosomal membrane so that exogenous drugs can enter the exosomes. However, at the same time, molecules in the exosomes may flow out[90]. Therefore, after electroporation of a mixture of exosomes and drugs, it is necessary to incubate at 37 °C to restore the exosomal membrane[83]. Sonication is also one of the methods for loading exosomes with drugs. After 6 cycles of sonication of a mixture of PTX and M1 macrophage-derived exosomes (30 s per cycle, with a 2-min cooling time after each cycle), PTX was shown to be successfully loaded into exosomes. Similar to electroporation, it is also necessary for a sonicated mixture to be incubated at 37 °C after sonication to restore the exosomal membrane[91]. In addition, dialysis can also be used to load drugs. In a study, a mixture of doxorubicin hydrochloride and exosomes derived from bone marrow MSCs was desalted and then dialyzed against PBS overnight to obtain doxorubicin-encapsulated exosomes[92].

There are differences in the drug-loading capacity and the size and morphology of obtained drug-exosome formulations. In a study on different methods of loading catalase into exosomes to treat Parkinson's disease, different amounts of catalase were found to be loaded into exosomes. Sonication and extrusion methods could load the most exosomes, followed by freeze–thaw cycles, but the coincubation method loaded the fewest exosomes[13]. Similarly, comparing the loading efficiency of incubation to that of electroporation and sonication, it was found that the efficiency of incubation was the lowest, while electroporation was higher than that of incubation but much lower than that of sonication[93].

Usually, the drug-loading efficiency of exosomes is less than 30%[13, 93], while that of other nanoparticles can reach more than 90%[94]. The methods for fabricating high drug-loading nanoparticles include pre-loading, co-loading and post-loading. In the pre-loading method, drug nanoparticles is first prepared, then coated with a layer of other materials. Drug-loaded nanoparticles can also be formed by the self-assembly of drug-conjugated macromolecules, which is called the co-loading method. In the post-loading method, usually porous nanoparticles is first formed, followed by mixing with drug[95].

Modification of exosomes with targeting ligands

The *in-vivo* biodistribution of drug-loaded exosomes is of great importance during cancer treatment. Specific delivery of anti-cancer drugs to cancer sites or cancer cells can significantly increase drug killing efficiency and prevent drug damage to normal cells. Exosomes which inherit the characteristics of donor cells, express ligands that naturally target certain recipient cells, and are eventually taken up by recipient cells[96]. Furthermore, the targeting ability of exosomes can also be improved by surface modification of exosome membrane.

The surface modification of exosome membrane could be achieved by direct modification and indirect modification. The indirect modification is referred to the engineering of cells that release exosomes. Cell engineering methods include genetic engineering, metabolic engineering and direct membrane engineering of the parent cells [97]. The most common method is genetic engineering. It includes viral and non-viral methods. Fusing the targeting peptide to the N-terminus of exosome-associated transmembrane protein Lamp2b, and followed by transfecting the encoded plasmid into cells would result in the display of targeting peptide on the membrane surface of isolated exosomes[98]. Through genetic engineering of HEK293 cells, the released exosomes

expressing IL3-Lamp2 could target the interleukin 3 receptor (IL3 receptor) which is highly expressed in Chronic Myeloid Leukemia cells. When compared with exosomes which were not modified by IL3, exosomes expressing IL3 were more tumor-targeted[99].

Direct modification methods could be achieved for induced targeting by chemical modification. Bio-orthogonal copper-free azide alkyne cyclo-addition (called click chemistry) was found to be able to conjugate ligands to the surface of exosomes. The cyclo (Arg-Gly-Asp-D-Tyr-Lys) peptide (c(RGDyK)) was able to bind to integrin αvβ3 in ischemic cerebral vascular endothelial cells. Conjugating (c(RGDyK)) to the surface of exosomes by click chemistry enabled the effective targeting of ischemic brain[100]. In addition, exosomes could be delivered to target sites using a magnetic field. Co-incubation of iron-loaded transferrin-conjugated Fe_3O_4 superparamagnetic nanoparticles (SPMNs) with exosomes enabled to form SPMN-exosomes by targeting the transferrin receptor on the exosome membrane[101].

Drug-loaded exosomes in cancer therapy

Because exosomes secreted by one type of cell can be taken up by another type of cell, it is believed that exosomes can be used as natural carriers for drug delivery. Drug-loaded exosomes can be used for the treatment of cancer, central nervous system diseases, inflammation, etc.[100, 102, 103]. Here, we mainly discuss the application of drug-loaded exosomes in the field of cancer therapy.

Gastric cancer

Gastric cancer is the fifth most common cancer type in the world, and has the third highest mortality rate[104]. Radiotherapy, chemotherapy and surgery are commonly used in the treatment of gastric cancer[105]. However, when chemotherapy is chosen, patients may have adverse reactions because not all drugs exclusively target the tumor site. Drug resistance often occurs, resulting in a poor prognosis. Therefore, considering that exosomes can be used as carriers for drug delivery, many studies have focused on the use of exosomes to target gastric cancer for treatment.

As mentioned above, the types of drugs that can be delivered by exosomes include small-molecule drugs, miRNAs, siRNAs, and proteins. It has been shown that HGF can promote the growth of gastric cancer[106], while HGF-specific siRNA can reduce HGF levels, thus inhibiting the growth of tumors. Zhang *et al*. applied exosomes to deliver HGF-specific siRNA to the tumor site, inhibiting tumor growth by suppressing the proliferation, migration and angiogenesis of a gastric cell line (SGC-7901)[79]. Similar to HGF, c-Met, as the receptor of HGF, is also highly expressed in gastric cancer, which accounts for the development of resistance to cisplatin, leading to a poor prognosis. Encapsulating c-Met-specific siRNA into exosomes extracted from the cell culture medium of HEK293-T cells and then delivering the exosomes to gastric cancer cells can reduce c-Met levels, thereby reducing resistance to cisplatin[104]. In addition, exosomes combined with photodynamic therapy (PDT) can be used to treat gastric cancer. Ce6 used in PDT can produce singlet oxygen under laser irradiation to induce cell apoptosis. Considering the poor compatibility of Ce6, Pan *et al*. synthesized a nanocomposite material, PMA/Au-BSA@Ce6, and loaded it into exosomes from patient urine to obtain Exo-PMA/Au-BSA@Ce6. Compared with free CE6 and PMA/Au-BSA@CE6, Exo-PMA/Au-BSA@CE6 accumulated more specifically in tumor sites and could significantly reduce the survival rate of gastric cancer cells (MGC-803 cells)[107]. Engineered tumor-targeting exosomes can also be obtained by plasmid transfection. Exosomes are transfected with plasmids expressing a fusion protein of internalized RGD (iRGD), and the resulting iRGD-Exos can target tumors. Recombinant methioninase (rMETase) can clear methionine, thereby inhibiting the G2 phase and tumor growth. A study found that rMETase-loaded iRGD-Exos (iRGD-Exos-rMETase) had a stronger inhibitory effect on tumors than rMETase and blank-Exos-rMETase[108].

Lung cancer

Lung cancer is a common type of cancer with a high mortality rate, and it can also be treated with chemotherapy, radiotherapy and surgery[109]. Lung cancer can be divided into small cell lung carcinoma (SCLC) and non-small cell lung carcinoma (NSCLC) according to histopathology. NSCLC is often treated with a combination of platinum drugs and paclitaxel[110]. However, free drugs and traditional nanocarrier drugs lack specificity and can cause side effects. The emergence of exosomes as carriers with the characteristics of low immunogenicity and a tumor-homing capability can significantly improve therapeutic effects. Therefore, using exosomes to deliver drugs to treat lung cancer has become a new strategy.

The targeting of exosome-drug formulations to lung cancer can be improved, by exploiting the specific binding between lung cancer cells and exosomes. For example, integrin beta 4 on the surface of exosomes (231-Exos) derived from breast cancer cells (MDA-MB-231) can specifically bind to surfactant protein C on the surface of lung cancer cells; that is, 231-Exos are enriched in the lungs. Therefore, miRNA-126, which has an inhibitory effect on lung cancer cells, can be loaded into 231-Exos to target and suppress lung cancer[111]. Appropriate modification of exosomes can also achieve a desired therapeutic effect. For example, exosomes were modified with anisamide-polyethylene

glycol (AA-PEG), as AA can bind to the sigma receptor overexpressed in lung cancer cells (3LL-M27 cells), so that paclitaxel encapsulated in the exosomes could be delivered to 3LL-M27 cells. PEG is beneficial for avoiding clearance and prolonging the circulation half-life of exosomes *in vivo* [112]. Folic acid (FA)-functionalized exosomes can also be used to target tumors. After intravenous injection of different formulations in a lung cancer model, the accumulation of the dye Alexa Fluor 750 (AF750) in the tumor site increased in the following order: AF750<ExoAF750<FA-ExoAF750. This suggests that FA functionalization can allow more exosomes to be taken up at the tumor site[113].

Brain cancer

Brain diseases, especially brain cancer, are seriously harmful to human health. The presence of the blood–brain barrier makes the treatment of brain cancer difficult. Many antitumor drugs are unable to cross the blood–brain barrier (BBB) and thus fail to exert therapeutic effects. Therefore, approaches to enable drugs to cross the blood–brain barrier has become a focus in brain cancer therapy. At present, nanodrug delivery systems, such as liposomes, can cross the blood–brain barrier and can be used to treat brain diseases[114]. However, they are biologically toxic as foreign substances and have poor stability[115]. Exosomes with the ability to cross the blood–brain barrier and low immunogenicity have become a new type of drug carrier and have attracted much attention.

It has been found that the cell source and administration route of exosomes have effects on the distribution of exosomes in the body. For example, after intravenous injection, exosomes mainly accumulate in the liver and spleen and exhibit little accumulation in the brain and tumors[76]. Therefore, in the treatment of malignant brain tumors, it is very important to enhance the targeting of exosomes to the brain so that drug-loaded exosomes can cross the blood–brain barrier to play a pharmacological role. The methods that have been found to enable drug-loaded exosomes to reach the brain include intranasal administration, the use of exosomes with natural brain-targeting capabilities, and exosome engineering (Fig. 2). Regarding the administration route, intranasal administration, as an administration route that effectively bypasses the blood–brain barrier, can enable drug-loaded exosomes to reach the brain. For example, curcumin-loaded exosomes administered intranasally can significantly inhibit the growth of brain tumors[116]. In addition, exosomes produced by certain cells have natural brain-targeting capabilities. Macrophage-derived exosomes can target brain endothelial cells through the interaction between the integrin lymphocyte function-associated antigen 1 (LFA-1) on exosomes and intercellular adhesion molecule 1 (ICAM-1) on brain endothelial cells[96, 117]. Exosomes derived from brain endothelial cells can also be loaded with antitumor drugs to treat brain cancer[118]. Reticulocyte-derived exosomes in the blood achieve brain-targeting through the interaction between transferrin and transferrin receptors[119]. Exosomes from brain-metastatic cancer cell lines (*e.g.*, SK-Mel-28) can also enter the brain[120, 121]. Exosomes without a natural brain-targeting ability can be modified to distribute in the brain. One approach is to engineer cells to produce exosomes that target the brain. In a study, dendritic cells were engineered to release exosomes expressing lamp2b because lamp2b can

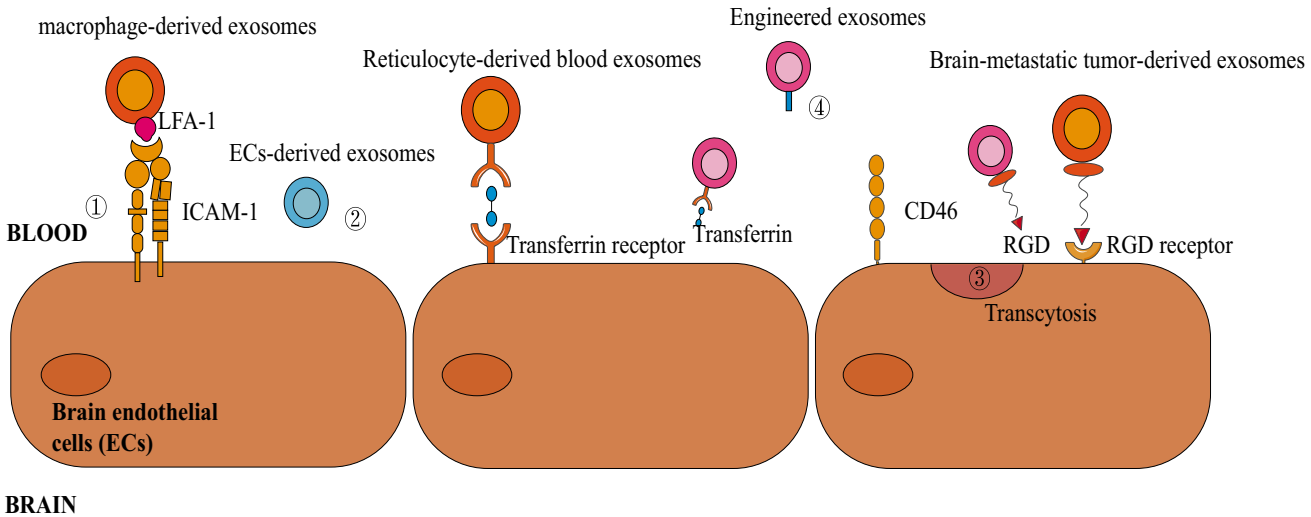

Fig. 2 Methods for Exosomes to Cross the bbb to Reach the Brain. Four types of Exosomes could Target Brain: (1) Exosomes from Certain Cells could cross the bbb through Ligand-receptor Interaction (such as lfa-1 and icam-1, transferrin and transferrin receptor, rgd and rgd receptor); (2) exosomes from brain endothelial cells have natural brain targeting capability; (3) brain-metastatic cancer cells could breach the bbb through transcytosis; (4) exosomes modified with ligands that target brain ecs could interact with receptors on ecs to cross the bbb

fuse with the neuron-specific RVG peptid; thus, exosomes could reach the brain[98]. Another approach is to directly modify exosomes after isolation. Exosomes modified with the RGERPPR (RGE) peptide can target gliomas that overexpress Neuropilin-1 (NRP-1) through the interaction between RGE and NRP-1[122]. Exosomes modified with the Cyclo (Arg-Gly-Asp-D-Tyr-Lys) peptide can also target glioblastoma that overexpresses αvβ3 integrin receptors[123].

Other cancers

In addition to gastric cancer, lung cancer, and brain cancer, which are described in detail above, other cancers can also be treated with drug-loaded exosomes. As reported previously, Anthos-loaded exosomes and paclitaxel-loaded exosomes can significantly inhibit the growth of ovarian cancer[124]. Compared with paclitaxel alone, paclitaxel-loaded M1 macrophage-derived exosomes have a stronger anti-breast cancer effect[91]. To target triple-negative breast cancer (TNBC), exosomes can be modified with a disintegrin, metalloproteinase 15 (binding to the integrin $\alpha_v\beta_3$)[125] and FA (binding to FA receptors overexpressed by TNBC cells)[126]. Similarly, exosomes can be used as carriers loaded with drugs to treat pancreatic cancer[127], prostate cancer[128], colorectal cancer[129], cervical cancer[88], chronic myelogenous leukemia[99] and so on and play an effective antitumor role.

In the process of applying drug-loaded exosomes as a cancer therapy, the accumulation of exosomes in the tumor site is very important for the loaded drug to exert its therapeutic effect. In addition to some exosomes having natural targeting capabilities, modification of exosomes to improve their targeting is often employed. This type of modification can allow exosomes to accumulate mainly in specific locations but may also cause an immune response. This is due to the presence of major histocompatibility complex molecules and costimulatory molecules on the exosomal membrane derived from dendritic cells, which can enhance the immune response[96, 130]. However, exosomes with a natural targeting ability also have the disadvantage of a low yield. Therefore, it is very important to choose a suitable cell source for exosomes and increase the production of exosomes to treat cancers.

Advantages and disadvantages of drug-loaded exosomes in cancer therapy

Due to the enhanced permeability and retention effect on tumor tissue, a nanodrug delivery system (NDDS) can pass through blood vessels to accumulate in tumors[131]; however, the NDDS is immunogenic and toxic[132]. As natural drug carriers, exosomes have many advantages in cancer therapy. First, exosomes, as vesicles produced by body cells, have superior biocompatibility and low immunogenicity[74]. Second, exosomes have natural or acquired targeting characteristics, allowing loaded drugs to accumulate in specific organs or tissues. For enabling targeting of tumors by exosomes, there are three approaches: exploiting a receptor-ligand interaction or antigen–antibody binding and targeting the microenvironment[133]. We also know that exosomes derived from tumor cells have tumor-homing properties. Cells tend to take up exosomes produced by the same cell type[134]. Therefore, tumor-derived exosomes usually accumulate at the tumor site[14]. In addition, the exosomal protein CD47 can bind to signal regulatory protein alpha, which triggers "don't eat me" signaling to prevent clearance by the mononuclear macrophage system, thus prolonging the half-life of exosomes[127]. Preinjection of an exosomal blockade agent can also be used to block the phagocytosis of exosomes by macrophages, prevent massive accumulation in the liver and spleen, and further allow engineered exosomes to reach the target site[135]. Due to the above advantages of exosomes, drug-loaded exosomes can significantly inhibit the growth of tumors compared to free drugs and nanocarrier-encapsulated drugs, such as liposomes-encapsulated drugs[127].

However, the application of drug-loaded exosomes in cancer treatment also has the defects of low drug-loading efficiency and a low yield. Both this and the low production of exosomes limit the clinical application of exosomes. To overcome these problems, researchers have performed a substantial amount of research and developed many ways to increase the production of exosomes. For example, culturing cells on a cell nanoporation chip and separating and loading exosomes simultaneously. Compared with the electroporation drug-loading method, this method increases the output of exosomes by 50 times[136]. Extrusion of exosomes with synthetic lipids can increase the number of exosomes by 6–43 times while maintaining the targeting property[137]. Some exosome-mimetic nanovesicles have been designed to increase yield. Serial extrusion of cells using nanometer-sized membranes or filters can produce a large number of exosome-mimetic nanovesicles. The output can be increased by 100 times[138, 139]. Furthermore, the emergence of artificial chimeric exosomes can also solve the problem of low yield of exosomes[140]. The artificial chimeric exosomes are prepared by integrating the cell membrane proteins of various cells into the phospholipid bilayer, which can ultimately possess multiple targeting capabilities from all parental cells and be produced in large scale. For instance, artificial chimeric exosomes produced using membrane proteins of red blood cells and MCF-7 cancer cells can not only express CD47 protein, avoiding being cleared by macrophages, but also inherit the targeting effect of cancer cells[141]. Although the above-mentioned exosome-mimetic nanovesicles are different from natural exosomes in some aspects, they can be a future developmental direction to overcome the weaknesses of exosomes.

In addition to the drug-loading efficiency and yield, the cell source of exosomes, exosome isolation methods, drug-loading methods and route of administration may all affect the therapeutic effect of drug-loaded exosomes[76]. All these aspects need to be considered to make the most favorable choice for treatment.

Conclusion and perspective

On the basis of many years of research, the composition, function, isolation and characterization of exosomes are relatively clear. Many studies have been performed on exosomes, and exosomes can be used as carriers for drug delivery. Exosomes, as a new type of drug carrier, have attracted extensive attention in the field of cancer therapy. Different antitumor effects are produced after drug loading with exosomes from different cellular sources with different compositions, yields, drug loading efficiencies and targeting capabilities; thus, any specific study must select appropriate exosomes. To treat cancer, it is often necessary to engineer exosomes so that the exosomes can accumulate at the tumor site through antigen–antibody binding, a receptor-ligand interaction, or targeting of the microenvironment. Moreover, in research on drug-loaded exosomes in cancer therapy, drug-loaded exosomes have been shown to have the advantages of relatively strong antitumor efficacy, low immunogenicity, a good targeting capability, and a long half-life *in vivo*. This makes drug-loaded exosomes more advantageous than traditional nanocarrier drugs. The best cell sources and the best drug-loading methods for the treatment of various cancers have not yet been fully elucidated. These points require further research in the future.

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Declarations

Conflict of interest All co-authors have no conflicts of interest.

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