

# Exosomes as Actively Targeted Nanocarriers for Cancer Therapy

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Yan Wang<sup>1,\*</sup>  
Yingru Zhang<sup>1,\*</sup>  
Gang Cai<sup>1</sup>  
Qi Li<sup>1,2</sup>

<sup>1</sup>Department of Medical Oncology & Cancer Institute of Integrative Medicine, Shuguang Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, People's Republic of China; <sup>2</sup>Academy of Integrative Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, People's Republic of China

\*These authors contributed equally to this work

**Abstract:** In recent years, it has been found that exosomes can be used as nanocarriers, which can be used in the treatment of tumors by carrying contents. The exosomes are derived from the secretion of the organism's own cells and are characterized by a phospholipid bilayer structure and a small particle size. These characteristics guarantee that the exosomes can carry a wide range of tumor drugs, deliver the drug to the cancer, and reduce or eliminate the tumor drug band. The toxic side effects were significantly eliminated; meanwhile, the therapeutic effects of the drug on the tumor were remarkably improved. This paper reviewed the strategies and drugs presented by different scholars for the treatment of tumors based on the drugs carried by exosomes.

**Keywords:** exosomes, nanocarriers, function, tumor therapy

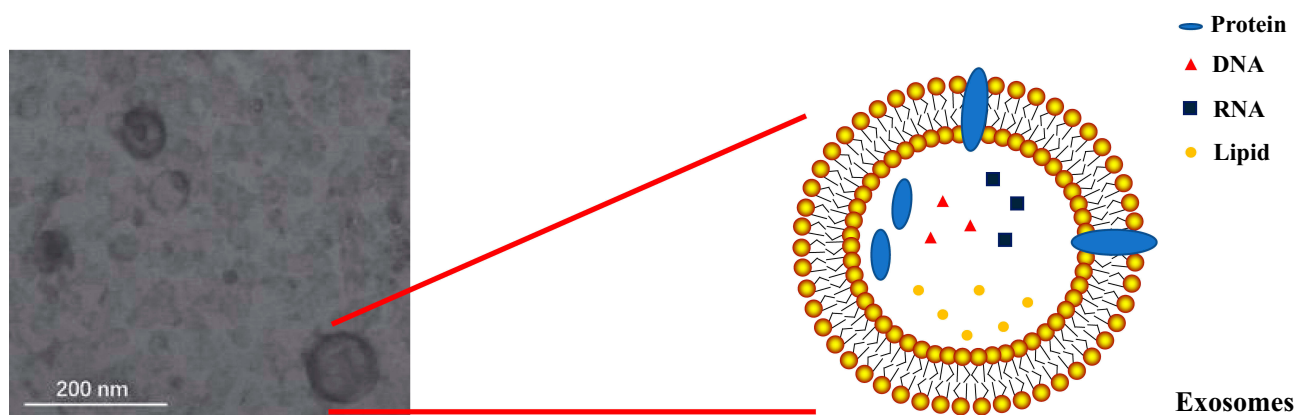
## Introduction

Exosomes are a class of natural nanoscale membrane vesicles formed by living cells through a series of regulatory processes, such as “endocytosis-fusion-efflux”.<sup>1</sup> In brief, exosomes were first discovered around 40 years ago.<sup>2</sup> In recent decades, people's understanding of exosomes has remarkably grown. At the beginning, it was thought that exosomes were such a path of cell excretion,<sup>3</sup> and further researches revealed that exosomes are also a medium participating in information exchange and material transportation between cells by carrying proteins,<sup>4</sup> lipids,<sup>5</sup> nucleic acids,<sup>6</sup> and other substances of host cells. Consequently, exosomes are used as a kind of nanocarrier to transport nucleic acids (such as miRNA<sup>7</sup>) or drugs (such as paclitaxel<sup>8</sup>) for the treatment of various diseases, such as tumors. The mechanism of better-utilizing exosomes and construct low-toxic or non-toxic granules with high-efficiency exosomes-loading, which were used in cancer treatment, has quietly become a research hotspot.<sup>9,10</sup> The present study systematically expounds exosomes, and summarizes the application of exosomes as nanocarrier-loaded drugs in tumor therapy, with the aim of providing a reference for future treatment of cancer.

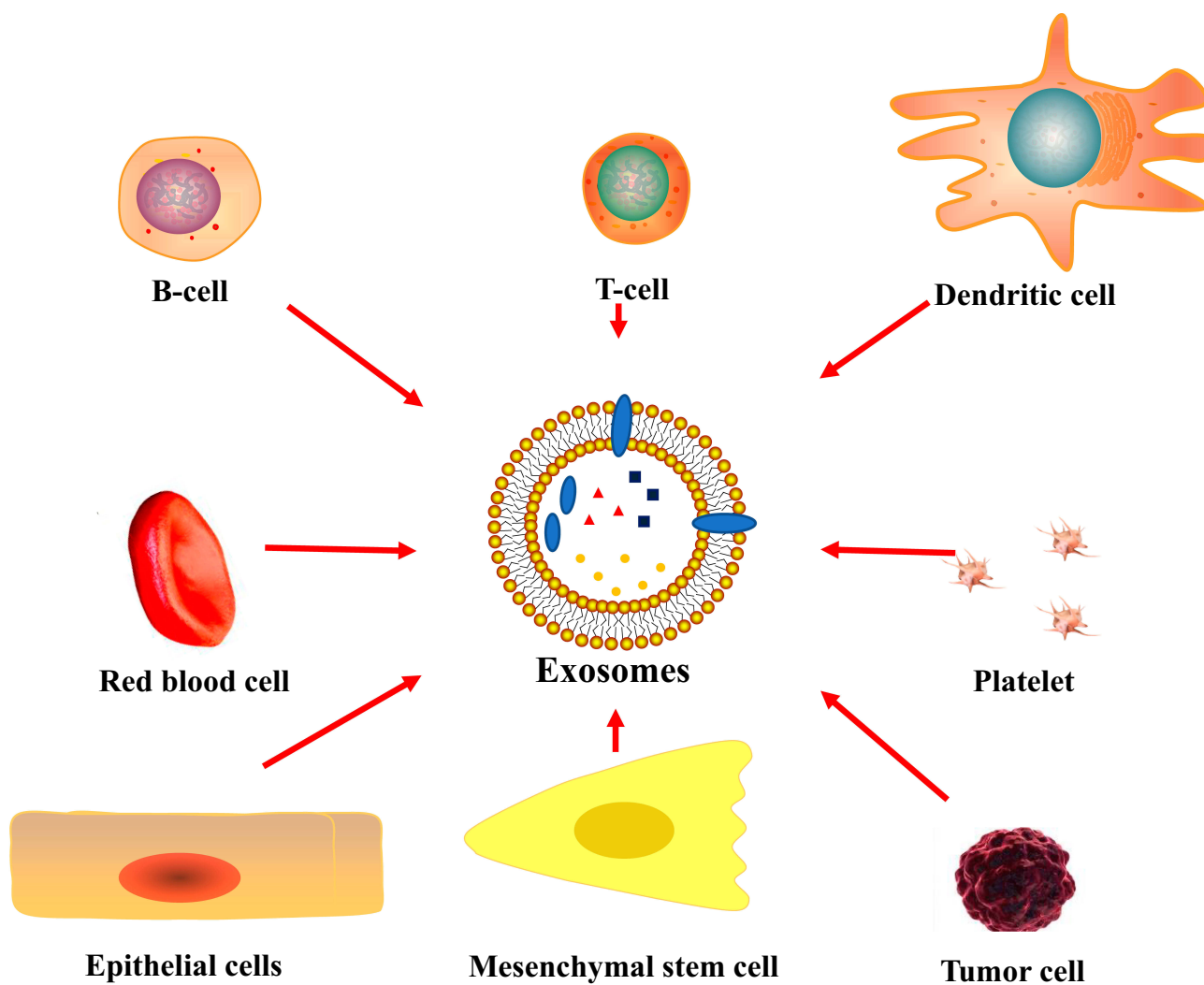
## Structure

Exosomes are a class of round-shaped lipid bilayer vesicles with a diameter of ranging from 30–150 nm, and in the image of exosomes EM, we can see that their shape is round-shaped and the distribution is monodisperse.<sup>11–14</sup> As early as 1981, Trams et al<sup>2</sup> discovered the existence of exosomes. Pan et al<sup>15</sup> reported the formation of exosomes via electron microscopy in 1985. Until 1987, the term “exosome” was first officially introduced by Johnstone et al.<sup>16</sup>

Correspondence: Qi Li  
Email qili@shutcm.edu.cn



**Figure 1** The ingredients of exosomes include multiple proteins, lipids, and nucleic acids, including RNA and DNA. And the scanning electron microscopy image of exosomes<sup>175</sup> was made by ourselves.

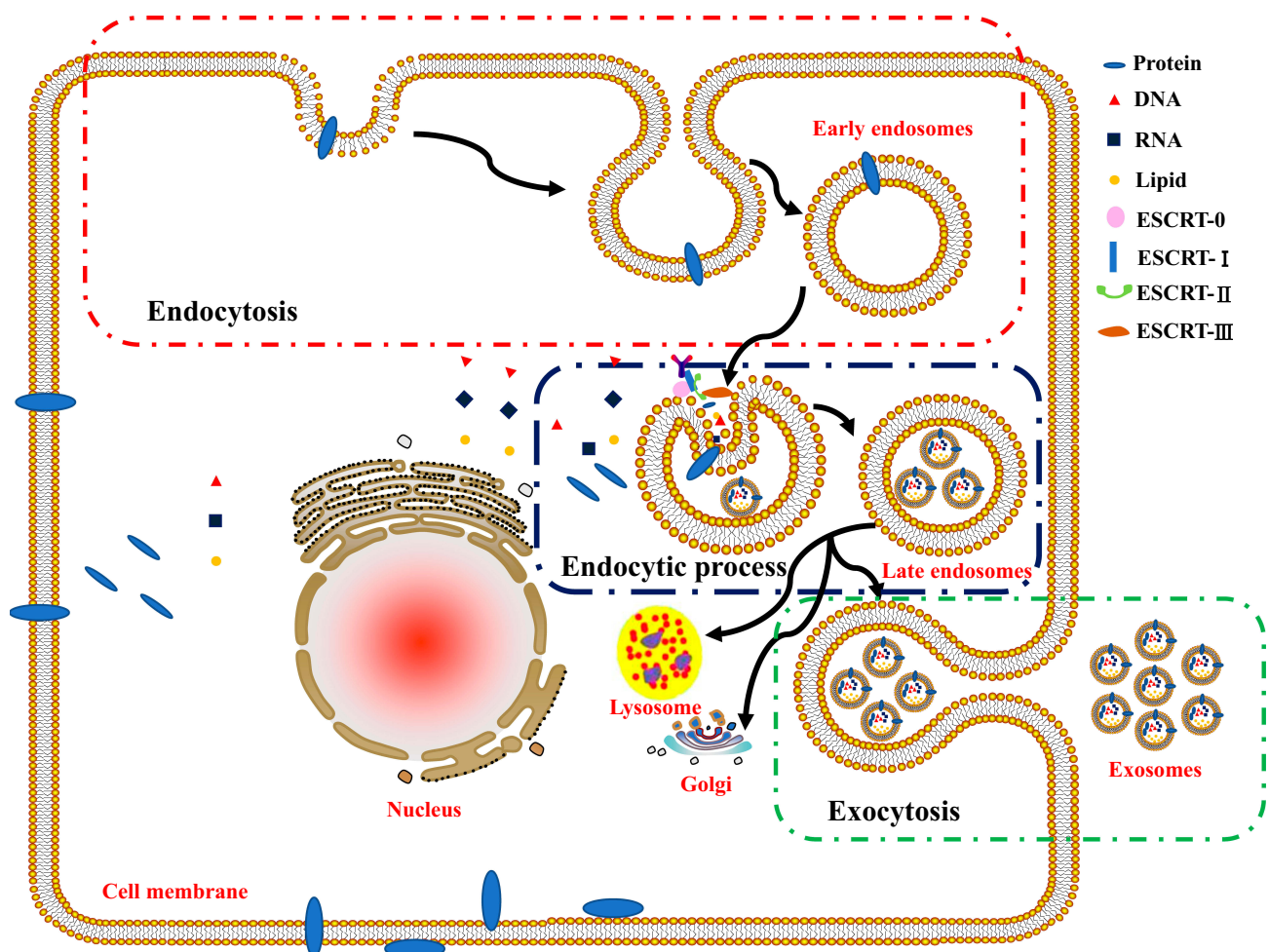


**Figure 2** Usually, exosomes can be naturally secreted by a variety of cells, such as T and B lymphocytes,<sup>31,32</sup> epithelial cells,<sup>33</sup> endothelial cells,<sup>34</sup> dendritic cells,<sup>35</sup> mesenchymal stem cells,<sup>36-38</sup> platelets,<sup>39,40</sup> tumor cells.<sup>41,42</sup>

The ingredients of exosomes (Figure 1) include multiple proteins,<sup>4,17</sup> lipids,<sup>5</sup> and nucleic acids, such as mRNA,<sup>18–20</sup> tRNA,<sup>21</sup> miRNAs,<sup>22–25</sup> LncRNA,<sup>26–28</sup> and DNA.<sup>6,29</sup>

Typically, almost all mammalian cells could secrete exosomes (Figure 2),<sup>30</sup> including T and B lymphocytes,<sup>31,32</sup> epithelial cells,<sup>33</sup> endothelial cells,<sup>34</sup> dendritic cells,<sup>35</sup> mesenchymal stem cells,<sup>36–38</sup> platelets,<sup>39,40</sup> tumor cells,<sup>41,42</sup> and act as transmitters and couriers in cellular crosstalk.<sup>43</sup> The source of exosomes is broad, and they can be found in several body fluids, such as tears,<sup>44</sup> nasal mucus,<sup>45</sup> saliva,<sup>46</sup> breast milk,<sup>47</sup> urine,<sup>48–50</sup> semen,<sup>51,52</sup> lymph,<sup>53</sup> and plasma,<sup>54</sup> etc. Sokolova et al<sup>55</sup> found that the size and integrity of the exosomes were strongly dependent on the storage conditions: the exosome diameter significantly decreased within 2 days at 37 °C and 4 days at 4 °C, but storage at –20 °C to –80°C can be stored for months to years.

It was revealed by pulse tracking and electron microscopy that exosomes were generated by endocytic pathway.<sup>56</sup> The specific generation process is as follows (Figure 3):<sup>57</sup> early endosomal stage, the cell membrane forms early endosomes through endocytosis; late endosomal stage: on the basis of the early endosomes, ESCRT-0 first bind to the specific receptors on the surface of the early endosomal membrane through the ubiquitination binding site, as well as selectively splicing part of the cytoplasm to form intraluminal vesicles by budding, ESCRT-I binds to ESCRT-0 and induces ESCRT-II to bind to ESCRT-I. Then, ESCRT-I is synergized with ESCRT-II to promote the formation of ILVs, followed by ESCRT-III shearing the bud of the neck. The ILVs are separated from the endosomal membrane, thereby releasing the ILVs encapsulating specific proteins, nucleic acids, and other substances into the endosomal cavity, as well as



**Figure 3** The process of exosomes is divided into three parts, namely endocytic process, endosome process, and exocytosis.<sup>56</sup> The endosome is stroked by endocytosis, and then matured into a late endosome containing multiple intraluminal vesicles,<sup>57</sup> which is multi-vesicle bodies (MVBs). The small vesicles secreted by MVBs to the extracellular membrane are exosomes. Some MVBs are degraded by lysosome phagocytosis, and a small part is degraded by Golgi and then recycled.<sup>58</sup>

completing the budding process, thereby forming mature late endosomes, and due to the late endosomes containing multiple ILVs, they are also called multi-vesicle bodies (MVBs).<sup>58</sup> Exocytosis: afterwards, some MVBs are degraded by fusion with lysosomes, while a number of them are fused with the plasma membrane, in which the internal vesicles of the MVBs are released into the extracellular medium as exosomes. Besides, there are some MVBs combined with the Golgi body for recycling.

## The Methods for Isolation and Identification of Exosomes

At present, the methods for isolation of exosomes are mainly divided into centrifugation,<sup>59</sup> precipitation,<sup>60</sup> ultrafiltration,<sup>61</sup> and immunoassay.<sup>62</sup> Their main purpose is to remove the mixed cells and their fragments and other small molecular impurities in the exosomes. Among those methods, centrifugation is a differential and a sucrose density gradient centrifugation method.<sup>63</sup> The principle of differential centrifugation<sup>64</sup> is based on the difference in sedimentation rates of different protein molecules, vesicles, cells, and cell debris in a homogeneous suspension. The sucrose density gradient centrifugation method<sup>65</sup> is based on the difference in density of each component in the sample. Under the action of centrifugal force, particles with low density are lifted upward, and particles with high density are sedimented downward. The density of enriched exosomes is at the range of 1.13~1.19 g/mL. The precipitation<sup>60,66,67</sup> is mainly a polyethylene glycol precipitation method. Because PEG is extremely hydrophilic, it can bind to the hydrophobic lipid bilayer and change the solubility or dispersibility of the exosomes to precipitate. Ultrafiltration method<sup>61,68</sup> uses the ultrafiltration membrane with the corresponding molecular weight cut off according to the size of the exosomes. Ultrafiltration technology is used to remove residual cells by centrifugation, in which a 0.22  $\mu\text{m}$  filtration membrane is used to remove cell debris, macromolecular vesicles, etc., and pure exogenous secretion is isolated. With any method mentioned above, the exosome can be separated. The cystic bodies with double membrane secreted by the cell membrane not only involve the component of the exosome, but vesicles and microvesicles (MVs) (with the diameter of 100nm–1000nm) dropped off from the cell membrane after becoming activated, injured or dead.<sup>69,70</sup> It is difficult to separate them from exosome with common separation methods; however,

there is a promising one, the emerging microfluidic system, a microscale separation based on the physical and biochemical properties, such as immune affinity, size and density, that can also achieve the innovative separation mechanism for acoustics,<sup>71</sup> electrophoresis<sup>72</sup> and electromagnetism.<sup>73</sup> With this technique, components of exosome and other microvesicles with different physical properties can be effectively separated. However, due to the lack of standardization and large-scale clinical sample testing, the microfluidic system has failed to be widely used.

While, the identification of exomes mainly focuses on the identification of morphological structure, size, number, and surface protein of exosomes.<sup>74</sup> Using electron microscopy,<sup>75</sup> we can clearly observe that the morphological structure of exosomes is mostly disc-shaped, which is composed of two layers of membranes, with light internal staining and deep external staining. The Nanoparticle Tracking Analysis can be used to measure and analyze the number and size of exosomes without destroying the structure and function of exosomes.<sup>76,77</sup> As a kind of Nanosight-related technique, the NTA technique is based on laser light scattering microscopy that can visualise and dynamically size populations of particles in the particle size range of 10 nm–1000 nm under a liquid state on an individual basis. And the Brownian motion of each and every particle is tracked separately but simultaneously using a CCD camera, from which a high-resolution plot of the particle size distribution profile is obtained. Jin et al<sup>78</sup> determined the size and concentration of exosomes through Nanosight Tracking Analysis by utilizing Zeta View PMX 110 according to previous protocol. Western blotting,<sup>79</sup> flow cytometry,<sup>80</sup> and mass spectrometry<sup>81</sup> can detect the type of exosome surface protein expression, in addition to its amount.

## Biological Functions of Exosomes

Previously, the understanding of exosomes was limited to transport some non-essential proteins and other molecules from the donor cells, thus, people originally thought exosomes are the only path of cellular excretion of waste.<sup>82</sup> With the increase of evidence, exosomes have been confirmed to play an important role in the body's physiological, pathological processes, such as carrying material, as well as exchanging information between local and distant cells.<sup>17,83,84</sup> Normally, these two roles of exosomes complement each other.



With the deepening of research, it is found that exosomes can be used as a biomarker<sup>85,86</sup> in clinical practice. Iaccino et al<sup>87</sup> studied exosome secreted by multiple myeloma and found that these exosomes express the immunoglobulin B-cell receptor, which binds to the unique type of binding peptide. The results of this study can be used as one of the biomarkers for the early diagnosis of multiple myeloma. What's more, exosomes play an important role in neural signal conduction,<sup>88</sup> immune response,<sup>89,90</sup> inflammation,<sup>91</sup> coagulation,<sup>92</sup> cell proliferation and differentiation,<sup>93</sup> tumor invasion and metastasis<sup>94,95</sup> and angiogenesis<sup>96</sup> etc.

Due to the basic characteristics of the stability of the lipid membrane structure encapsulating the genetic information material, the widespread distribution in body fluids, and the ease of availability, and their nanoscale and great biocompatibility, exosomes could be used as a potential nanocarrier for clinical tumor therapy. In this review, we focused on the application and progress of exosomes as nanocarriers in tumor therapy.

## Comparing with Conventional Nanocarriers

In general, traditional nanocarriers can be divided into two categories (Tables 1 and 2): organic nanocarriers (such as liposomes,<sup>97–99</sup> micelles,<sup>100–102</sup> etc.) and inorganic nanocarriers (eg, mesoporous silica nanoparticles,<sup>103–105</sup> graphene,<sup>106,107</sup> magnetic nanoparticles,<sup>108–110</sup> gold nanoparticles,<sup>111–113</sup> quantum dots,<sup>114,115</sup> and layered double hydroxides,<sup>116–118</sup> etc.). Compared with conventional nanocarriers, exosomes have several their own advantages and enormous potential in clinical tumor treatment.

First of all, due to the phospholipid bilayer structure, exosomes could carry drugs stably to avoid enzymes degrading drugs and extend the half-life of drugs during delivering, and their membranes could mix well with target cells. Correspondingly, the bioavailability of the loaded drug improved as well.

Then, compared with traditional drug carriers like liposomes, the immunogenicity, and toxicity of exosomes were poor. Moreover, we cannot ignore their petite body. The nanosize of exosomes allows them to be extravasated

**Table 1** The Summary of Inorganic Nanocarriers

Group	Advantages	Disadvantages	References
Carbon nanotube	Have superior transmembrane ability due to their tubular structure; plus their large specific surface area, they can be loaded with high molecular weight drugs; in addition, they are chemically stable, easy to modify, and can be linked to various biomolecules to improve targeting. At the same time, the carbon nanotube complex can prolong the circulation time of the drug in the body	It has poor dispersibility in most polar solvents, is easy to agglomerate, and has certain cytotoxicity. At the same time, its length is long, the surface has many impurities, lacks functional groups, and cannot be directly applied	[176–180]
Mesoporous silica	Have good biocompatibility, regular pore structure and controllable pore size, large specific surface area, easy surface modification, easy synthesis and good stability	There is drug leakage, and the carrier is not highly targeted	[181–184]
Magnetic nanoparticles	Have excellent biocompatibility, small size, low toxicity, heat treatment, surface modification, and superior magnetic orientation	Has low drug loading and low bioavailability, and it is difficult to control the size of industrialized nano-iron oxide. In clinical applications, the heat during hyperthermia is difficult to control	[185–187]
Gold nanocarriers	Have high electron density, dielectric properties and catalysis. They can be combined with various biomacromolecules for biomedical and detection. Surface-enhanced Raman scattering properties can be used to detect biomolecule levels in vivo and accurately diagnose lesions. Increasing the length-to-length ratio of the plasmon resonance of the nano-gold longitudinal surface to the near-infrared region, and converting it into thermal energy or singlet oxygen, exerting the near-infrared thermal effect	The surface modification method of gold nanocarriers is not abundant, and then the cost is also high. At the same time, the biosafety of nanogold is still questioned	[188–190]

**Table 2** The Summary of Organic Nanocarriers

Group	Advantages	Disadvantages	References
Liposomes	Have good biocompatibility and biodegradability; they can be used to embed hydrophilic, hydrophobic and amphiphilic drugs, have wide applicability, and have relatively high drug loading; surface-modified liposomes not only improve drug utilization, but also reduce toxic side effects	Phospholipids in liposomes are easily oxidized, poor overall stability, low industrial reproducibility, and difficult sterilization treatment	[191,192]
Micelles	Have a low critical micelle concentration, high stability in body fluids, high drug loading rate, and chemically modified surface to introduce other functional groups	Have a limited range of use and are limited to the entrapment of hydrophobic drugs	[193,194]
Dendrimer	Due to its monodisperse size, surface functionalization, water solubility and versatility of internal cavities, dendrimers have become a drug delivery carrier with great potential, and the surface is easily chemically modified while simultaneously functioning with multiple functions. The combination of molecules such as imaging contrast agents, targeting ligands and drugs allows people to prepare multifunctional drug delivery platforms	The synthesis process of dendrimers is cumbersome, the synthetic products are difficult to separate and purify, and the synthesis cost is high, so it cannot be widely used in industrial production, and it is difficult to extract on a large scale	[195,196]
Exosomes	Have the following characteristics: they can stably carry drugs to avoid enzymatic degradation of drugs and prolong the half-life of drugs during delivery; extremely high bioavailability; extremely low immunogenicity and toxicity; can spread in tumor tissues, and Pass the human blood brain barrier	Hard to extract on a large scale	[8,9,28,122,197]

in tumor vessels and spread in tumor tissue to treat tumors. Thus, exosomes can also overcome some physiological barriers (eg, blood-brain barrier).<sup>119–122</sup> Another advantage is that when the drug is loaded in exosomes, the efficacy of the drug is enhanced.

However, the only drawback is the difficulty of large-scale extraction. However, a number of scholars have discovered that other sources of exosomes can also be as same as human exosomes, can be utilized as anti-tumor drug carriers, and successfully applied to the treatment of cancer. Munagala et al<sup>9</sup> found that bovine milk-derived exosomes can be used as nanocarriers for the delivery of chemotherapeutic/chemopreventive agents for the treatment of lung cancer, and can increase antitumor activity. Katakowski et al<sup>123</sup> exploited the characteristics of exosomes capable of loading miRNAs, in which they used exosomes as nanocarriers carrying miRNAs to treat gliomas after loading miR-146b.

## The Function of Exosomes in Tumor

Tumor-derived exosomes are involved in pathogenesis and microenvironmental establishment of cancer. To explore the specific function of exosomes in tumors, it is necessary to

explore its complexity and functional heterogeneity. Exosomes have been widely proved to play an important role in the formation of tumor microenvironment,<sup>124</sup> tumor invasion and metastasis,<sup>125,126</sup> angiogenesis<sup>127</sup> and tumor immunity.<sup>128</sup> There is no doubt that exosomes secreted by cancers are one of the main biological mediators of tumor progression but some exosomes have been found to inhibit the occurrence and development of tumors. Which kind of exosomes to be choose is the most critical point in treatment. Logozzi et al<sup>129</sup> suggest that nanomaterials getting to tissues are scavenged by macrophages that then release them through exosomes. Iessi et al<sup>130</sup> devised a novel strategy involving the use of exosomes as carriers which is purified from culture supernatant of macrophages isolated from peripheral blood of healthy donors. The exosome delivery system showed to actually enhance the tumoricidal effect of Acridine Orange (AO), by increasing the exposure time of the biological targets. This evidence suggests that the exosomes release by macrophages potentially useful material. Exosomes have been detected in a variety of body fluids such as urine, saliva, bile, and blood. In tumor patients, the content of exosomes has become an important information to judge the occurrence and development of tumors.<sup>131–134</sup>

Therefore, preventing the secretion and circulation of tumor exosomes can effectively inhibit the progression of cancer. The results show that the high exosomes content in tumor tissues may be related to the acid anoxic microenvironment<sup>124,135</sup> and  $\text{Ca}^{2+}$ .<sup>136</sup> The presence of an anoxic environment results in an acidic microenvironment, then results in an increased secretion of exosomes. Some studies showed that the exosomes of  $\text{CD63}^+$ ,  $\text{CD9}^+$  and  $\text{ALIX}^+$  increased by five times when the calcium content increased. It is therefore a useful option to suppress acidic microenvironments or to use  $\text{Ca}^{2+}$  channel blockers before using exosomes as carriers to treat tumors.

## Application of Exosomes as Nanocarriers in Tumor Treatment

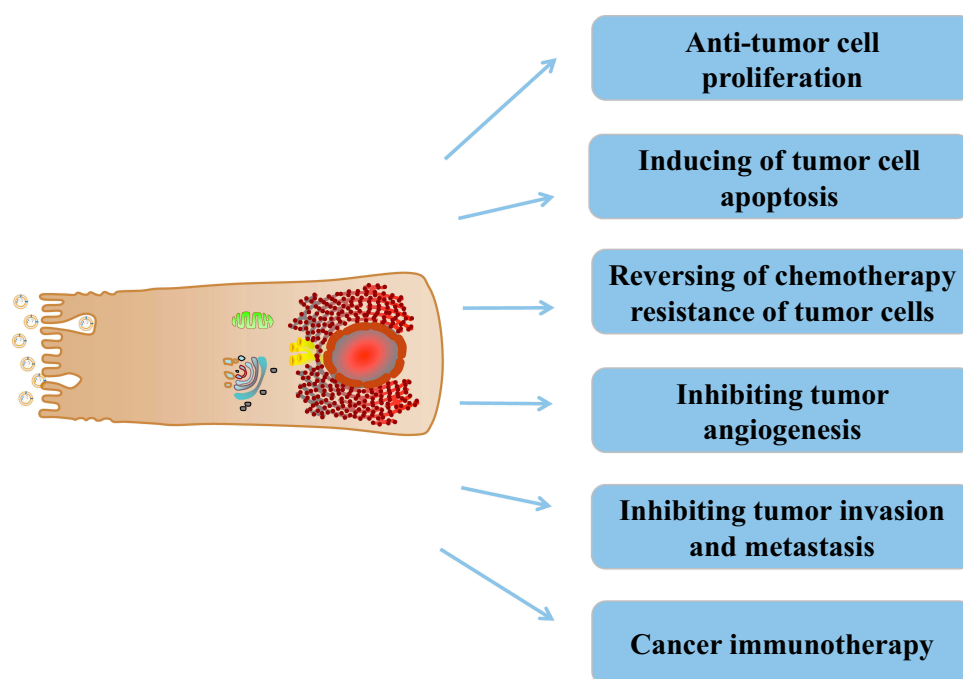
It was revealed that utilizing exosomes as nanocarriers to load antitumor drugs or siRNAs into exosomes may cause them possess the following characteristics, such as enhancing the efficacy of drugs, as well as expanding the current therapeutic range; increasing the bioavailability of drugs; targeting; being non-toxic or low-toxic, etc.<sup>137–139</sup> We know that, Hadla et al<sup>140</sup> made exosomal doxorubicin (ExoDOX) by electroporation, in which ExoDOX is more potent than free doxorubicin. Qi et al<sup>141</sup> developed a dual-functional exosome-based superparamagnetic nanoparticle cluster using

exosomes as a targeted drug carrier for the treatment of tumors. At present, the anti-tumor effect of exo-drugs mainly represents the occurrence and development of tumors by inhibiting cell proliferation, inducing apoptosis, inhibiting drug resistance, inhibiting tumor angiogenesis, and inhibiting cell invasion and metastasis. Simultaneously, exo-drugs can also treat tumors by regulating immunity (Figure 4).

Although some unanswered problems and methodological challenges remain, this rapidly advancing field have already provided important insights into the relevance of EVs in the clinical setting. The great clinical impact in nanomedicine of exosomes has been explored,<sup>142–144</sup> The clinical research data have already demonstrated that exosomes secreted by immune cells stimulate the immune system and can be exploited as antitumor vaccines.<sup>145,146</sup> Several clinical trials involving the use of extracellular vesicle-based delivery are ongoing, for example for the treatment of lung cancer and melanoma, that may become part of an immunotherapy approach that has great potential for patients with advanced cancers.<sup>147</sup>

## Anti-Tumor Cell Proliferation

Compared with the mono drug action, the exosomes could enhance the anti-tumor cell proliferation characteristic of the drug via generating the exo-drugs with drug loading. F. Aqil et al<sup>10</sup> mixed 10% of curcumin ethanol with



**Figure 4** The anti-tumor effect of exo-drugs mainly represents the occurrence and development of tumors by inhibiting cell proliferation,<sup>10,123,148–150</sup> inducing apoptosis,<sup>151–154</sup> reversing chemotherapy resistance of tumor cells,<sup>155,158</sup> inhibiting tumor angiogenesis,<sup>119,159</sup> and inhibiting cell invasion and metastasis.<sup>11,160</sup> Simultaneously, exo-drugs can also treat tumors by regulating immunity.<sup>161–166</sup>

acetonitrile to form exosomal curcumin (ExoCUR) with a drug loading rate of 18–24%. In vivo experiments showed that ExoCUR was absorbed compared to free curcumin, and the rate increased by 3–5 times; in vitro experiments showed that ExoCUR inhibited tumor cell proliferation, which was also significantly enhanced. More meaningful, ExoCUR has no systemic toxicity. Similarly Aqil et al<sup>148</sup> used milk-derived exosomes to encapsulate berry anthocyanins to form ExoAnthos, which demonstrated that in vivo experiments Exo preparations may increase drug stability, uptake ratio, and half-life, allowing Anthos to be slowly released from exosomes to increase the anti-cancer effect in ovarian cancer.

Moreover, the exosomes play the role as the carrier for doxorubicin to generate the Exo-Dox, which functioned on MFC-7 cells and significantly inhibited the cell growth under Yang et al<sup>149</sup> exploration. This effect is significantly superior to that of free doxorubicin. After combining Exo-Dox with heat-stress, Exo-Dox-HS showed better inhibition than Exo-Dox, and the content of doxorubicin in cells was higher as well. Subsequent examines using exosomes to load drugs not only revealed their own advantages, but also other technologies (eg, heat-stress) were employed to further enhance the anti-tumor efficacy of Exo-drugs.

In addition, the exosomes could also contribute to the other mediator function, such as the miR-146b and iRG peptides. Katakowski et al<sup>123</sup> used the plasmid expressing miR-146b to transfect the marrow stromal cell to secrete M146-exosomes (M146-exo). M146-exo may inhibit the growth of gliomas by inhibiting the expression of EGFR, thereby treating tumors. Tian et al<sup>150</sup> first modified the murine immature dendritic cells to produce exosomes carrying iRGD peptides on the surface, namely iRGD-Exos. iRGD-Exos was then loaded with DOX by electroporation to make iRGD-Exos-Dox. iRGD-Exos-Dox can target  $\alpha v$  Integrin-positive tumor cells and can specifically bind to MDA-MB-231 cells and inhibit cells proliferation.

## Inducing of Tumor Cell Apoptosis

Studies have shown that exosomes also have a good effect in inducing tumor cell apoptosis. At present, exosomes can induce tumor cell apoptosis in two ways. One is to carry a content to directly induce apoptosis of tumor cells. When exosomes are combined with drugs that induce apoptosis, the ability of the drug to induce apoptosis is enhanced, and the toxicity of normal tissue cells can be remarkably reduced. Interestingly, the manufactured EXO-drugs possess some features, such as targeting and pH sensitivity. For example, Srivastava et al<sup>151</sup> creatively combined gold nanoparticles

with apoptosis-inducing doxorubicin and made Nano-Dox for the first time. Afterwards, Nano-Dox was incubated with exosomes to make Exo-GNP-Dox. Exo-GNP-Dox has the following characteristics: it can be effective up taken by tumor cells, while it is hardly up taken by normal cells; it may cause tumor cell apoptosis by activating caspase-9, as well as inducing DNA damage; it also can induce ROS in tumor cells, and interfere with mitochondrial membrane potential, eventually leading to increased DNA damage and apoptosis in cancer cells, thereby protecting normal cells. And Xu et al<sup>152</sup> screened exosomes expressing miR-29c and acted on bladder cancer BIU-87 cells. It was revealed that miR-29c carried by this exosome can down-regulate BCL-2 and MCL-1 to induce cell apoptosis.

There are also exosomes that, after being secreted by cells, the proteins carried by the exosomes themselves can induce the apoptosis of tumor cells without needing to re-design the exosomes. For instance, Zhu et al<sup>153</sup> purified the exosomes secreted by NK cells and obtained NK-cell-derived exosomes (NK-Exo), which contained perforin and granzymes. That's to say, NK-Exo active apoptosis pathway of glioblastoma via increased formation of apoptosome and caspase-3 activation with the help of perforin and granzymes. At the same time, Hosseini et al<sup>154</sup> immobilized SEB to produce EXO/SEB. Then, they used EXO/SEB in estrogen receptor-negative breast cancer cells to induce apoptosis by inhibiting the expression of anti-apoptotic gene BCL-2, as well as promoting the expression of the Bax and Bak genes.

## Reversing of Chemotherapy Resistance of Tumor Cells

Several tumors are resistant to chemotherapeutic drugs after chemotherapy. For example, drug-resistant cells produce drug-resistant proteins, such as P-glycoprotein,<sup>155</sup> which can drain the chemotherapeutic drugs out of the cells, reduce the concentration of chemotherapeutic drugs in cells, and eventually cause cell resistance. At present, EXO-drug can reverse the chemotherapy resistance of tumor cells mainly through two ways, one way is to reduce the expression of drug resistance proteins in drug-resistant cells. Munoz et al<sup>156</sup> found that in glioblastoma multiforme cells, due to the increase of miR-9, the expression of P-glycoprotein was also increased, resulting in tumor cell resistance to temozolomide. They utilized exosomes secreted by mesenchymal stem cells to carry anti-miR-9, targeting resistant cells, as well as inhibiting the expression of drug resistance proteins, in order to enhance the sensitivity



of tumor cells to chemotherapy drugs, thereby reversing the resistance of tumor cells.

And the other way is to increase the sensitivity of drug-resistant cells to chemotherapy drugs. Kim et al<sup>157</sup> loaded Paclitaxel (PTX) into exosomes by ultrasonic technology, so that exosomes encapsulated PTX and made PTX-exosome (exoPTX). Compared with free PTX, they found that exoPTX increased the toxicity of drug-resistant cells by 50-fold, indicating that exoPTX can enhance the sensitivity of drug-resistant cells to chemotherapeutic drugs, thereby improving the therapeutic effects of the drug on drug resistant cancers. Lou et al<sup>158</sup> used miR-122 to transfect adipose tissue-derived mesenchymal stem cells, secreted exosomes coated with miR-122, and exosomes were used for the treatment of hepatocellular carcinoma by improving the sensitivity of liver cancer cells to chemotherapeutic drugs.

### Inhibiting Tumor Angiogenesis

As we know, the continuous growth of tumors depends on the supply of tumor blood vessels, and inhibiting tumor angiogenesis has a very appropriate inhibitory effect on tumor growth. In fact, in the process of tumor angiogenesis, exosomes themselves are involved in this process, and studies have found that exosomes can promote tumor angiogenesis by carrying miRNAs such as miR-25-3p. For example, Zeng et al<sup>96</sup> found that miR-25-3p was transfected into endothelial cells by exosomes in colon cancer cells, and miR-25-3p up-regulated expression of VEGFR2, p-AKT, p-ERK, and down-regulated ZO-1, occludin and Claudin5 by silencing KLF2 and KLF4, thereby inducing angiogenesis.

Based on this research, it was found that exosomes can inhibit tumor angiogenesis by carrying siRNA such as HGF siRNA. Such as the research of Zhang et al.<sup>159</sup> They loaded HEK293T-derived exosomes with hepatocyte growth factor siRNA (HGF siRNA), and found that the exosomes could inhibit the expression of HGF/VEGF in SGC-7901 cells, thereby inhibiting the growth of tumor blood vessels. The exosomes can also inhibit the growth and metastasis of tumor cells. In addition, Yang et al<sup>119</sup> found that siRNAs of vascular endothelial growth factor (VEGF) were transfected into exosomes derived from brain endothelial bEND.3 cells to produce exosome-delivered siRNAs. In the brain cancer model of zebrafish, exosome-delivered siRNAs can inhibit the expression of VEGF in brain cancer cells through the blood-brain barrier, thereby inhibiting tumor angiogenesis.

### Inhibiting Tumor Invasion and Metastasis

Studies have shown that exosomes themselves are involved in the process of tumor invasion and metastasis. QinLan et al<sup>94</sup> found that in colorectal cancer, M2 macrophage-derived exosomes carrying miR-21-5p and miR-155-5p are transferred to colorectal cancer cells, which bind to the BRG1 coding sequence and inhibit BRG1 expression, thereby promoting the invasion and metastasis of colorectal cancer. Chen et al<sup>95</sup> also demonstrated that tumor invasion and metastasis are related to exosomes.

On this basis, people think that when exosomes are loaded with therapeutic substances, they can also inhibit the metastasis of tumors. Kamerkar et al<sup>11</sup> presented exosomes carrying siRNA or shRNA to specifically target the oncogenic KRASG12D, which can inhibit the metastasis of pancreatic cancer and prolong the survival rate by reducing the expression of KRAS gene in a variety of pancreatic cancer mouse models. In addition, Shim et al<sup>160</sup> transfected miR-143 into mesenchymal stem cells to secrete exosomes loaded with miR-143 to make exosome-formed miR-143. Then, they applied exosome-formed miR-143 to osteosarcoma cells, which inhibited the metastasis of osteosarcoma cells.

### Cancer Immunotherapy

After engineering exosomes, a specific tumor antigen was expressed on the surface. Such exosomes can activate immune cells in vivo and inhibit the growth of tumors expressing antibodies corresponding to their antigens, so as to achieve efficient treatment of tumors. Cho et al<sup>161</sup> investigated whether the autologous or allogeneic exosomes express their specific tumor antigen human MUC1 on the surface, and they can activate immune cells and inhibit the growth of MUC1-expressing tumors, in order to achieve the tumor treatment. These scholars first constructed the recombinant lentivirus pLXIN-muc1, and then infected mouse-derived CT26 and TA3HA cells with pLXIN-muc1 to obtain two types of cells, including CT26-MUC1 and TA3HA-hMUC1. These cells can be secreted, CT26-hMUC1 exosomes and TA3HA-hMUC1 exosomes. These two exosomes can promote the proliferation and activation of immune cells, and effectively inhibit the growth of tumors expressing MUC1. Morishita et al<sup>162</sup> transfected a plasmid vector fused to streptavidin-lactocin with murine melanoma B16BL6 cells, engineered an exosome expressing streptavidin-lactocin (SAV-LA), and then used SAV-LA-expressing exosomes (SAV-exo). After integration with biotinylated CPG-DNA, CPG-DNA-modified SAV-exo was made, which was CpG-SAV-exo. They found that CPG-

sav-exo can effectively activate mouse dendritic dc2.4 cells compared with CPG and SAV-exo, thereby enhancing the tumor antigen presentation ability of dc2.4 cells, indicating that CPG-sav-exo has a proper anti-tumor effect.

Some exosomes are secreted and purified from the cells in the body, and the tumor antigen is directly expressed on the surface without modification. Besides, the exosomes suppress the growth of the tumor by mediating the immune reaction. For example, in the study of liver cancer conducted by Rao et al<sup>163</sup> human hepatocellular carcinoma HepG2 cell-derived exosomes were isolated and purified, which could induce dendritic cells to produce a strong immune response. Thus, they could inhibit the growth of the tumors by increasing the number of T lymphocytes, elevating the levels of interferon- $\gamma$ , as well as decreasing the levels of interleukin-10 (IL-10) and tumor growth factor- $\beta$  (TGF- $\beta$ 1) in tumor sites. Bu<sup>164</sup> et al loaded the exosomes from dendritic cells into chaperone rich cell lysates (CRCLs) and made DEX (CRCL-GL261)-DCs, in which in vivo experiments showed that DEX (CRCL-GL261)-DCs could promote the proliferation of cytotoxic T cells, leading to increase their activity, stimulate the production of anti-tumor factors IL-2 and IFN $\gamma$ , and eventually inhibit tumor growth. Wang et al<sup>165</sup> found that exosomes derived from CD40 ligand gene-modified 3LL tumor cells have a stronger immune effect and can induce more IFN- $\gamma$  and IL-2 secretion after dendritic cells become more mature. Furthermore, Gehrman et al<sup>166</sup> produced an exosome that not only expressed the antigen ovalbumin (OVA), but also loaded the invariant NKT (iNKT) immune cell ligand  $\alpha$ -galactosylceramide ( $\alpha$ GC), by a series of immune responses, such as activation and proliferation of iNKT, NK, gamma delta ( $\gamma\delta$ ) T cells ( $\gamma\delta$  T cells), as well as proliferating OVA-specific CD8+ T cells, in order to inhibit tumor growth.

In addition to these common chemical treatments for tumors, it has also been found that tumors can be treated by exosome combined with physical therapy. Altanero et al<sup>167</sup> found that exosomes carrying magnetic nanoparticles were effectively engulfed by tumor cells by endocytosis. Under the induction of external alternating magnetic fields, magnetic nanoparticles produced high temperatures, causing tumor cell ablation.

## Conclusion

Chemotherapy and targeted therapy can hardly achieve the desired curative effect and result in the process of oncotherapy due to various defects, and patients may also have various uncomfortable physiological responses due to large amounts of drug intake. Exosome has been

widely used as a biologically carrier of therapeutic materials, as its great drug loading capacity, high specificity and low immunogenicity are natural advantages that other nanometer materials do not have. The application of further customized exosome drugs in oncotherapy provides an important platform for the research and development of next-generation antineoplastic drugs. However, such drugs should be produced in a larger scale at more strictly controlled quality for application. In addition to rigorous toxicity observation, the drugs also need to be supported by the ultimate clinical tests. As far as the current research progress is concerned, the application of exosomes, in addition to low yield, needs to be studied in the following aspects: 1, The structure and mechanism of action of exosomes have not been thoroughly studied, and further research is needed; 2, Exo-drugs are targeted, but the stability of their targeting has not been studied in depth, and there may be off-target phenomenon. 3, Exosomes from different sources carry a variety of substances from donor cells, leading to exogenous secretion. There are differences between the bodies, and it is necessary to standardize the use of exosomes. 4, In some studies, the researchers use tumor cell-derived exosomes. While, exosomes in from tumor patients contain tumor support components which could cause tumor changes in recipient cells or organisms.<sup>168-171</sup> Cossetti et al<sup>171</sup> found that exosomes are capable of transferring substances of tumor cells to recipient cells. Plasma-derived exosomes need to identify and remove tumor support components during the purification process. In view of this, animal-derived exosomes are more or less problematic, and Pedro Perez-Bermudez et al have already found exosomes in food,<sup>172</sup> such as milk, fruits, vegetables, etc. They not only have been isolated exosomes from these foods by centrifugation, but also found that exosomes in milk may be able to act on the human body. For example, milk-derived exosomes can affect the body's immune function<sup>173</sup> by carrying proteins, and can also regulate the intestinal barrier function.<sup>174</sup> Perhaps we can use milk as a source of exosomes.

Even if these problems exist, with the breakthrough of research, it is just around the corner to use exosomes as nanocarriers for the treatment of tumors.

## Abbreviations

EM, electron microscope; miRNA, micro ribonucleic acid; mRNA, messenger ribonucleic acid; tRNA, transfer ribonucleic acid; miRNA, microRNA; LncRNA, long noncoding

RNA; DNA, deoxyribonucleic acid; ESCRT, endosomal sorting complexes required for the transport; ILVs, intraluminal vesicles; MVBs, multi-vesicle bodies; PEG, polyethylene glycol; TSG101, tumor susceptibility gene 101; ELISA, enzyme-linked immunosorbent assay; NTA, nanoparticle tracking analysis; EGFR, epidermal growth factor receptor; Dox, doxorubicin; RGD, Arg-Gly-Asp; ROS, reactive oxygen species; NK cells, natural killer cells; SEB, staphylococcal enterotoxin B; PTX, paclitaxel; VEGFR, vascular endothelial growth factor receptor; IL-2, interleukin-2; IFN $\gamma$ , interferon- $\gamma$ .

## Data Sharing Statement

The datasets generated and analyzed during the present study are available from the corresponding author on reasonable request.

## Author Contributions

All authors contributed to data analysis, drafting or revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

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## Disclosure

Authors declare no conflicts of interest for this article.

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