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**Roles and application of exosomes in the development, diagnosis and treatment of gastric cancer**

Guan XL *et al.* Roles and application of exosomes

## Abstract

As important messengers of intercellular communication, exosomes can regulate local and distant cellular communication by transporting specific exosomal contents and can also promote or suppress the development and progression of gastric cancer (GC) by regulating the growth and proliferation of tumor cells, the tumor-related immune response and tumor angiogenesis. Exosomes transport bioactive molecules including DNA, proteins, and RNA (coding and noncoding) from donor cells to recipient cells, causing reprogramming of the target cells. In this review, we will describe how exosomes regulate the cellular immune response, tumor angiogenesis, proliferation and metastasis of GC cells, and the role and mechanism of exosome-based therapy in human cancer. We will also discuss the potential application value of exosomes as biomarkers in the diagnosis and treatment of GC and their relationship with drug resistance.

**Key Words:** Exosomes; Gastric cancer; Immune regulation; Diagnosis; Cancer therapy

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**Core Tip:** Exosome-mediated transport plays an important role in the invasion and metastasis of gastric cancer (GC). In this review, we focus on the immunoregulatory role of exosomes and the relationship between exosomes and GC, with special attention to their role in the growth, invasion, metastasis, and therapeutic resistance of GC, as well as their potential clinical application value as biomarkers and therapeutic targets, and the mechanism and clinical application prospects of exosomal immunotherapy for human tumors.

## **7** INTRODUCTION

Gastric cancer (GC) is the fifth most commonly diagnosed cancer and the fourth leading cause of cancer death worldwide, with an incidence of 5.6%. Although the current level of treatment has been greatly improved, the mortality rate is still as high as 7.7%<sup>[1]</sup>. Metastasis, as the main cause of death in patients with GC, is caused by a multistep molecular cascade reaction inside and outside tumor cells and stromal cells. Exosome-mediated transport plays an important role in the invasion and metastasis of tumor cells<sup>[2]</sup>.

<sup>4</sup> Exosomes are bilayer lipid vesicles with a diameter of 30-100 nm, which are a subgroup of extracellular vesicles (EVs). These vesicles are released into extracellular space by a large number of cells and play a key role in the initiation and development of intercellular signalling networks. EVs include microvesicles, ectosomes, exosomes, membrane particles, apoptotic vesicles and many other types. Many cell types including tumor cells, epithelial cells, mast cells, fibroblasts, stem cells, and immune cells such as macrophages, monocytes, dendritic cells (DCs), B and T lymphocytes, and natural killer cells (NK), can secrete exosomes<sup>[3]</sup>. Exosomes are composed of lipid bilayers, and their main components are proteins, nucleic acids [mRNA, microRNAs (miRNAs) and DNA], amino acids, lipids and metabolites<sup>[4]</sup>. These cargos are selectively packaged in exosomes. Conventional markers of exosomes include CD63, tumor susceptibility gene 101 protein (TSG101), ALG2-interacting protein X (ALIX) and proteasome component HSC10. Exosomes are produced by the endolysosomal pathway<sup>[5]</sup>.

Exosomes can transmit information between tumor cells or to other normal cells, thus participating in complex intercellular communication. They also participate in a variety of cellular processes related to cancer development and drug resistance, showing the dual characteristics of promoting and inhibiting cancer.

In this review, we focus on the immunoregulatory role of exosomes and the relationship between exosomes and GC, with special attention to their role in the growth, invasion, metastasis, and therapeutic resistance of GC, as well as their potential clinical application value as biomarkers and therapeutic targets, and the mechanism and clinical application prospects of exosomal immunotherapy for human tumors.

## **BIOGENESIS OF EXOSOMES**

The biogenesis of exosomes includes four main stages: Initiation, endocytosis, formation of multivesicular bodies (MVBs) and exosome secretion<sup>[6]</sup>. The biogenesis of exosomes begins with the inward budding of the plasma membrane, forming a small intracellular body called endosome<sup>[7]</sup>. With the maturity of these early endosomes, intraluminal vesicles (ILVs) containing late endosomes will be formed under the control of endosomal sorting complex required for transport (ESCRT). These late endosomes containing ILVs are also called MVBs<sup>[8]</sup>. MVBs are formed by inward invagination of the endosomal limiting membrane, which causes MVBs to contain multiple ILVs. ILVs are eventually secreted as exosomes with a diameter of approximately 40 to 160 nm through exocytosis and the fusion of MVBs with the plasma membrane<sup>[9]</sup>. Rab GTPase mediates the intracellular transport of MVBs<sup>[6]</sup>. Exosomes are absorbed in neighboring cells through direct fusion, endocytosis, or interactions between proteins and recipient cells, and then transmit the information contained to target cells<sup>[10]</sup> (Figure 1).

The mechanism regulating the formation of MVBs is mainly driven by ESCRT. ESCRT is a complex composed of ESCRT-0, ESCRT-I, ESCRT-II, ESCRT-III and related proteins<sup>[11]</sup>. Other studies have found the existence of ESCRT independent mechanisms, mainly including syndecan-syntenin-ALIX protein, Rab protein family, the ceramide pathway, p53 state, intracellular Ca<sup>2+</sup> level, high level of heparanase and pH<sup>[2]</sup>. The exosomal protein ALIX promotes endosomal membrane budding and abscission as well as exosomal cargo selection through interaction with syndecan. Ceramide-rich lipid domains and tetraspanin CD63 outside membrane cells are essential for the formation of ILVs<sup>[12]</sup>.

## **IMMUNOMODULATORY EFFECTS OF EXOSOMES**

Exosomes act as transporters in the process of immune cell-cell communication and participate in the regulation of immunity. The exosomes secreted by APCs are rich in major histocompatibility complex class (MHC)-I/II and costimulatory molecules, which

directly present peptide antigens to specific T cells and induce their activation<sup>[13]</sup>. The regulation of immunity by exosomes mainly depends on tetraspanin-related proteins, such as integrins, immunoglobulin superfamily receptors and growth factor receptors, immune-related noncoding RNAs (ncRNAs), and other immune molecules expressed on exosomes, such as MHC and costimulatory molecules<sup>[14]</sup>. In summary, exosomes from tumor and immune cells can play related immunomodulatory roles by transferring signals related to immune stimulation or immunosuppression (Table 1).

#### *DC-derived exosomes*

DC is the primary antigen-presenting cell in the immune system and plays a role in initiating and maintaining T-cell-mediated responses. DC-derived exosomes contain intercellular adhesion molecule 1 (ICAM-1), which can interact with lymphocyte function-associated antigen-1 (LFA-1), bind to lymphocytes, and act as a ligand for ICAM-1 expressed on CD8<sup>+</sup> DCs, thereby activating T cells and promoting T lymphocyte proliferation<sup>[15]</sup>. Due to the mature state of DCs, DCs acquire immunostimulatory and inhibitory properties, which depend on the expression levels of costimulatory molecules (CD80 and CD86), MHC and coregulatory molecules [such as programmed cell death-ligand 1 (PD-L1) and PD-L2]<sup>[16]</sup>.

Because both mature DCs and immature DCs secrete exosomes, DC-derived exosomes are expected to have two phenotypes. It is known that immature DCs strongly release exosomes, and the number of exosomes gradually decreases with the maturation process. However, the exosomes released by mature DCs seem to have stronger antigen presentation ability to T cells than those released by immature DCs<sup>[17]</sup>. DCs play an important role in tumor immunity. DC-derived exosomes activate CD4<sup>+</sup> and CD8<sup>+</sup> T cells and induce an antitumor immune response through endogenous interleukin (IL)-2 and exosomal CD80 *in vivo*<sup>[18]</sup>.

#### *T lymphocyte derived exosomes*

T cells are mainly divided into two types according to phenotype, including CD4<sup>+</sup> helper T cells and CD8<sup>+</sup> cytotoxic T lymphocytes (CTLs)<sup>[19]</sup>. Exosomes isolated from CD4<sup>+</sup> helper T cells express T-cell markers [CD4, TCR, LFA-1, CD25 and Fas ligand (FasL)] and exosome-related proteins, which are involved in the CTL reaction and antitumor immune response<sup>[20]</sup>.

Regulatory T cells (Tregs)-derived exosomes are thought to contribute to immunosuppression mediated by CD25, CD73 and CTLA-4. On the other hand, the presence of CD39 and CD73 contributes to the inhibition of Tregs through the production of adenosine, an anti-inflammatory mediator<sup>[3]</sup>. Exosomal IL-35 targets T cells and B cells to induce infectious tolerance<sup>[21]</sup>. Treg-derived exosome-mediated Let-7d secretion inhibits type 1 T helper (Th1) cell proliferation and cytokine release by inhibiting the production of interferon- $\gamma$  mediated by cyclooxygenase-2<sup>[22]</sup>.

#### ***Macrophage-derived exosomes***

Macrophages have dual activities, M1 cells have proinflammatory effects and play an important role in antitumor immunity, and they are tumor-suppressing cells. M2 macrophages have anti-inflammatory and immunosuppressive activities and are tumor-promoting cells. Tumor-associated macrophage (TAM)-derived exosomes usually exhibit an M2-like phenotype. They lack cytotoxic activity, provide growth factors for cancer cells, and have immunosuppressive activity<sup>[23]</sup>. In the case of *Mycobacterium tuberculosis* infection, macrophage-derived exosomes both initiate a protective immune response and promote prior BCG immunization<sup>[24]</sup>. Exosomes released by TAMs can induce an imbalance in Treg/Th17 cells by transferring miR-29a-3p and miR-21-5p to CD4<sup>+</sup> T cells, thus directly creating an immunosuppressive microenvironment and promoting cancer progression<sup>[25]</sup>. Exosomes can also be used as transmitters to deliver antigens to immune cells and enhance the immune response. Exosomes released by macrophages act as transmitters to deliver antigens to DCs in a ceramide-dependent manner, thereby enhancing the CD4<sup>+</sup> T-cell immune response<sup>[26]</sup>.

### ***Mast cell-derived exosomes***

Mast cell-derived exosomes contain MHC class II, LFA-1, CD86 and ICAM-1. Mast cells can display mitosis activity on B and T lymphocytes *in vitro* and *in vivo* by secreting exosomes, and regulate the proliferation of B and T cells<sup>[27]</sup>. BMC-exosomes partially promoted CD4+ T cells proliferation, and BMC-exosomes promoted the proliferation and differentiation of Th2 cells between exosomes and T cells *via* ligation of OX40L and OX40<sup>[28]</sup>. Exosomes mediate the information exchange between mast cells and nerves, and exosomes derived from mast cells can regulate neuroimmunity at the local site of acupuncture<sup>[29]</sup>.

### ***NK cell-derived exosomes***

Studies have found that NK-derived exosomes express both NK cell markers (such as NKG2D, CD94, perforin, granzyme, and CD40L) and exosome-specific markers (such as TSG101, CD81, CD63, and CD9), all of which are involved in cytotoxicity and immune responses<sup>[30]</sup>. Exosomes derived from NK cells contain the tumor suppressor miR-186, which can effectively inhibit tumorigenic potential and transforming growth factor (TGF) $\beta$ -dependent immune escape mechanisms<sup>[31]</sup>.

### ***Tumor-derived exosomes***

Tumor-derived exosomes (TDEs) play a critical role in tumor growth by influencing different immunomodulatory mechanisms such as antigen expression, immune activation, immunosuppression, immune surveillance, and intercellular communication. TDEs can activate the immune response. Exosomes from tumor cells present new antigens and/or MHC- peptide complexes to activate NK cells or macrophages directly, or activate T cells *via* DCs<sup>[32]</sup>. TDEs also showed strong immunosuppressive responses. Exosomes polarize TAMs, induce tumor-associated neutrophils, regulate T-cell differentiation and function, inhibit DC maturation, suppress NK cell activity and induce myeloid suppressor cells (MDSCs), thereby exerting immunosuppressive effects<sup>[33]</sup>.



T cells are potential targets for TDEs to exert immunosuppression. Human TDEs can inhibit the IL-2-mediated proliferation of CD4 and CD8 T cells, and exosomes directly inhibit the killing function of NK cells in a manner independent of T cells<sup>[34]</sup>. TDEs can selectively induce T-cell apoptosis through FasL or inhibit T-cell receptor signalling by decreasing the expression of CD3-<sup>[35]</sup>. Exosomes derived from GC can induce apoptosis of Jurkat T cells in a time- and dose-dependent manner by mediating the degradation of the PI3K proteasome and the activation of caspases 3, 8 and 9<sup>[36]</sup>.

<sup>10</sup> Exosomes derived from cancer cells contain PD-L1, which can inhibit T-cell function and promote tumor growth. GC-derived exosomes effectively induce the production of PD-L1+ TAMs and impair CD8+ T-cell function *via* IL-10, and this immunosuppressive activity can be effectively enhanced by inducing programmed cell death 1 (PD-1) signalling<sup>[37]</sup>.

Mesenchymal stem cells (MSCs) treated with exosomes derived from GC cells promote the phagocytosis of macrophages through the nuclear factor kappa-beta (NF-<sup>9</sup>κB) signalling pathway, promote the secretion of proinflammatory factors, and promote the activation of CD69 and CD25 on the surface of T cells, thus enhancing the ability of MSCs to activate immune cells and maintain the inflammatory environment<sup>[38]</sup>.

MDSCs are immature suppressor cells that have the ability to promote tumor progression. Exosomes released by cancer cells can regulate the activation and expansion of MDSCs and enhance their immunosuppressive function<sup>[35]</sup>. Exosomes may also regulate immunity by influencing gene expression and signalling pathways in recipient cells, mainly through miRNA transfer. Exosomes secreted by cancer cells transfer miR-212-3p to DCs, which inhibits the expression of the MHC II transcription factor RFXAP in DCs, leading to decreased expression of MHC II and promoting the immune escape of cancer cells<sup>[39]</sup>. Exosomal miR-451 is not only an indicator of poor postoperative prognosis in GC patients, but is also associated with increased Th17 distribution in GC. The redistribution of miR-451 from cancer cells into infiltrating T cells during hypoglycaemic therapy can enhance Th17 differentiation by enhancing mechanistic target of rapamycin (mTOR) activity<sup>[40]</sup>.

<sup>3</sup> Therefore, exosomes play both immune-activating and immunosuppressive roles in cancer. The effect of immune activation mainly depends on the antigen presentation of exosomes, while the immunosuppressive effect of exosomes mainly depends on the ligands, proteins and miRNAs they carry, which inhibit the activity of cytotoxic T cells or increase the number of immunosuppressive cells<sup>[41]</sup>.

### **EXOSOMES AND GC DEVELOPMENT**

Exosomes can influence recipient cells through autocrine and paracrine signalling. First, exosomal proteins can influence the cells that release exosomes through the autocrine pathway. Second, exosomal DNA itself affects cell survival. Exosomes can also regulate the tumor microenvironment through paracrine mechanisms. Exosomes derived from host cancer cells can activate receptors or transfer proteins and RNAs to recipient cells, thereby affecting the tumor microenvironment or altering the biological phenotype of recipient cells<sup>[41]</sup>.

Tumour-derived microvesicles can be transmitted as messengers between GC cells and influence several important processes of malignant progression of GC, including angiogenesis, tumor migration, the establishment of pre-metastatic niche (PMN) and epithelial-mesenchymal transformation<sup>[42,43]</sup> (Table 2, Figure 2). GC cells can establish the PMN through various mechanisms, including immunosuppression, matrix remodeling, angiogenesis, mesenchymal transformation and organotropy<sup>[33]</sup>.

#### ***Exosomes and angiogenesis***

Angiogenesis is closely related to the occurrence, invasion and metastasis of tumors by providing oxygen and nutrients, and plays an important role in the progression of GC. Exosomes can regulate the characteristics of endothelial cells to promote angiogenesis. Exosomes derived from GC cells promote tumor angiogenesis by activating endothelial cells<sup>[44]</sup>. YB-1 plays a key role in exosomes promoting GC angiogenesis by upregulating angiogenic factors in endothelial cells treated with exosomes<sup>[45]</sup>. GC-derived exosomal <sup>2</sup>miR-519a-3p activates the MAPK/ERK pathway by targeting DUSP2, thereby causing

M2-like polarization of macrophages, inducing angiogenesis and promoting the formation of an intrahepatic premetastatic niche to accelerate liver metastasis of GC<sup>[46]</sup>.

It was found that X26nt expression was significantly increased in GC and GC-derived exosomes. Exosomal X26nt reduces vascular endothelial cadherin expression by directly binding to VE-cadherin mRNA in human umbilical vein endothelial cells, thereby increasing vascular permeability. *In vivo* experiments have shown that X26nt can promote angiogenesis in a mouse subcutaneous tumor model<sup>[47]</sup>. GC-derived exosomes mediate circular RNAs (circRNAs) delivery, and circ29, as a sponge of miR-29a, promotes angiogenesis by regulating the vascular endothelial growth factor (VEGF) pathway in endothelial cells<sup>[48]</sup>.

Studies have shown that exosomes derived from MSCs can promote or inhibit tumor angiogenesis through the Akt (protein kinase B)/eNOS pathway, the ERK1/2 pathway or miRNA transport<sup>[49]</sup>. Other scholars have found that exosomes loaded with hepatocyte growth factor (HGF) small interfering RNA can inhibit the growth and angiogenesis of GC cells and the growth rate of blood vessels<sup>[50]</sup>.

### *Exosomes and GC growth*

**Cancer cell-derived exosomes and GC growth:** The absence of some exosomal molecules may increase cell viability and promote proliferation. Gastrin-1 is a tumor suppressor protein. The deletion of gastrokinin 1 in exosomes can promote the proliferation of GC cell lines<sup>[51]</sup>. TRIM3 knockdown in serum exosomes of GC patients promotes the growth and metastasis of GC *in vitro* and *in vivo* by regulating stem cell factors and epithelial-to-mesenchymal transition regulatory factors<sup>[52]</sup>. GC-derived exosomes significantly increase the phosphorylation level of NF-κB in macrophages, and activate macrophages in human peripheral blood mononuclear cells by activating NF-κB to increase the level of proinflammatory factors, thus promoting the proliferation and migration of tumor cells<sup>[53]</sup>.

The extracellular signal-regulated kinase (MAPK/ERK) signalling pathway is mainly involved in the development and progression of tumors and is regulated by exosomes.

Tumor cell-derived exosomes partially activated the MAPK/ERK and PI3K/Akt pathways in SGC7901 and BGC823 GC cells. Exosomes containing CD97 activate the MAPK signalling pathway in SGC-7901 GC cells, which can promote cell proliferation and invasion. Exosomal miRNAs may be involved in the activation of CD97-related pathways<sup>[54]</sup>. ZFAS1 is a newly identified long ncRNA (lncRNA) that exists in exosomes and can be transmitted through them. Overexpression of ZFAS1 promotes the proliferation and migration of GC cells<sup>[55]</sup>.

MiRNAs are the most abundant and important biomolecules in exosomes, which play a key role in tumor regulation and may be related to tumor development, metastasis and prognosis. In the early stage of tumorigenesis, the downregulation of antitumor miRNA in cancer cells and the insufficient supplementation of exosomal miR-101 in the microenvironment of residential cells will stimulate the development of GC<sup>[56]</sup>. The level of exosomal miR-423-5p in the serum of GC patients and in the supernatant of GC cell culture was significantly increased, and miR-423-5p inhibited the expression of fusion protein inhibitory factor, promoting the proliferation and migration of GC cells<sup>[57]</sup>. GC cells induce pericytes to transform into cancer-associated fibroblasts (CAFs) through exosome-mediated BMP transfer and activation of the PI3K/AKT and MEK/ERK pathways, thereby inducing malignant progression of cancer<sup>[58]</sup>. There are abundant let-7 miRNAs in both the intracellular and extracellular environments of the metastatic GC cell line AZ-P7a, and AZ-P7a cells release let-7 miRNAs into extracellular environments through exosomes to support gastric carcinogenesis<sup>[59]</sup>.

**Microenvironment-derived exosomes and GC growth:** The tumor microenvironment includes MSCs, endothelial cells, tumor-associated fibroblasts, TAMs, pericytes, extracellular matrix, signalling molecules, *etc.* Tumor microenvironment-derived exosomes are also important for the progression of GC. Exosomes derived from bone marrow MSCs promote the growth of GC SGC7901 cells by activating Hedgehog signaling pathway<sup>[60]</sup>. Exosomal miRNA-34 derived from CAFs inhibit the proliferation and invasion of GC cells *in vitro* and inhibit tumor growth *in vivo*<sup>[61]</sup>. <sup>2</sup> M1 macrophage-



derived exosomes containing miR-16-5p can trigger T-cell immune responses and inhibit tumor formation *in vivo* and *in vitro* by reducing the expression of PD-L1<sup>[62]</sup>.  
1 Macrophage-derived exosomes can serve as carriers to deliver exogenous miR-21 inhibitors to BGC-823 GC cells, promoting cell migration and inhibiting cell apoptosis<sup>[63]</sup>.

### *Exosomes and GC invasion and metastasis*

**Cancer cell-derived exosomes and GC invasion and metastasis:** During tumor cell metastasis, the cells adhere to the stroma and migrate into the blood, reaching the premetastatic niche and then causing secondary tumors. Studies have shown that bioactive substances carrying exosomes, such as proteins, miRNAs, or lncRNAs, may be functional signals to induce tumor growth and metastasis in GC cells.

GC-derived exosomes induce increased neutrophil-autophagy through the HMGB1/TLR4/NF- $\kappa$ B signalling pathway, thus promoting the migration of GC cells<sup>[64]</sup>. Exosomal circSHKBP1 sponged miR-582-3p increased the expression of HUR, enhanced the stability of VEGF mRNA and inhibited the degradation of heat shock protein 90, thus promoting the progression of GC<sup>[65]</sup>. The expression of exosomal TGF- $\beta$ 1 and the proportion of Treg cells in the draining lymph nodes were significantly correlated with the pathological stage and lymph node metastasis of GC<sup>[66]</sup>. Exosomes are specific to the recipient cell type and are prone to organotropic metastasis. Organotropism is caused by primary GC cells actively selecting PMNs in a specific remote microenvironment. Exosomal epidermal growth factor receptor is transmitted from GC cells to Kupffer cells and hepatic stellate cells to effectively activate HGF by inhibiting the expression of miR-26a/b. In addition, HGF binds to c-MET receptors on cancer cells and copromotes liver-specific metastasis<sup>[67]</sup>.

In addition to lymph node metastasis, peritoneal metastasis is also a main type of metastasis in advanced GC patients. Cancer-derived exosomes induce increased expression of adhesion molecules in mesothelial cells, which is a necessary condition for peritoneal metastasis of GC. GC-derived exosomes are internalized into mesothelial

cells, which can significantly promote the adhesion between mesothelial cells and GC cells. After internalization into GC cells and malignant pleural effusion, <sup>5</sup> the expression of adhesion-related molecules such as fibronectin 1 and laminin gamma 1 in mesothelial cells increased, which further promotes the migration of GC cells<sup>[68]</sup>.

The intact mesothelium is the protective barrier of the peritoneum. GC-derived exosomes promote peritoneal metastasis through peritoneal fibrosis and mesodermal barrier breakdown. Upregulation of p-ERK in peritoneal mesothelial cells leads to mesothelial-to-mesothelial transition, which in turn leads to GC-derived exosomes damaging peritoneal mesothelial cells and causing peritoneal metastasis of GC<sup>[69]</sup>. This finding supports that exosomes play a key role in mediating peritoneal metastasis.

**Microenvironment-derived exosomes and GC invasion and metastasis:** CAFs partially promote the migration of GC cells through matrix metalloproteinase (MMP)11, and the CAFs-derived exosomal miR-139 can inhibit the progression of GC by decreasing the expression of MMP11<sup>[70]</sup>. MSC-exosomes activate the AKT signalling pathway, which then enhances the migration and invasion of GC cells by inducing epithelial-mesenchymal transformation, increases the expression of mesenchymal markers of GC cells, and enhances the tumorigenicity of GC cells *in vitro*<sup>[71]</sup>. M2 macrophage-derived exosomes remodel the cytoskeleton to support gastric cell migration<sup>[72]</sup>. The lncRNA PCGEM1 is specifically expressed in exosomes derived from hypoxia-cultured GC cells (HGCs). PCGEM1-rich exosomes derived from HGCs can promote the invasion and migration of GC cells in normoxic culture<sup>[73]</sup>. These studies all suggest that exosomes from the tumor microenvironment are also involved in GC metastasis.

## **THE APPLICATION OF EXOSOMES IN GC**

### ***Applications as biomarkers in GC***

The existence and content of exosomes, as well as their detectable characteristics in body fluids, make the diagnosis based on exosomes considered to be the best method

for noninvasive diagnosis, and therefore exosomes can be used as biomarkers for disease diagnosis and treatment (Table 3).

Serum exosomal miRNAs have been identified as potential biomarkers for GC. Studies have found that the expression levels of miR195-5p, miR10b-5p, miR296-5p and miR20a-3p in the exosomes of GC serum samples are significantly increased, and patients with high expression of miR10b-5p or miR296-5p have worse prognosis<sup>[74]</sup>. These studies suggest that exosomal miRNAs can serve not only as novel noninvasive diagnostic markers, but also as potential GC prognostic biomarkers.

ncRNAs are some of the most abundant RNAs in exosomes. Exosomes contain some ncRNAs with biological functions, and the membrane structure of exosomes significantly improves the stability of exosomal ncRNAs. These characteristics make exosomal ncRNAs suitable as biomarkers for diagnosis and prognosis<sup>[75]</sup>. The plasma LINC00152 level in patients with GC is significantly higher than that in healthy controls, and one of the possible mechanisms for its stable existence in the blood is the protection of exosomes. Studies have shown that the specificity and sensitivity of plasma LINC00152 in the diagnosis of GC are 85.2% and 48.1%<sup>[76]</sup>.

Exosomal TRIM3 can be used as a biomarker for the diagnosis of GC, and exosome transport of TRIM3 may provide a potential treatment for GC<sup>[52]</sup>. Studies on biomarkers of early GC have found that two exosomal lncRNAs specific to early GC, lncUEGC1 and lncUEGC2, are significantly upregulated in patients with early GC and in exosomes derived from GC cells. lncUEGC1 is expected to be a stable, highly sensitive and noninvasive biomarker for the diagnosis of early GC<sup>[77]</sup>.

Lymph node and peritoneal metastasis are associated with a poor prognosis in GC patients. Some scholars have found that the expression of exosomal TGF- $\beta$ 1 in GC patients is related to pathological stage and lymph node metastasis<sup>[66]</sup>. Researchers identified five highly expressed miRNAs (miR-320c, miR-1202, miR-1225-5p, miR-4270, and miR-1207-5p) in malignant ascites, peritoneal lavage fluid and culture supernatants. Among them, miR-21 and miR-1225-5p are related to serosal invasion of GC, and may be used as biomarkers of peritoneal recurrence after GC surgery<sup>[78]</sup>.

### *Exosomes and cancer therapy*

The membrane of exosomes protects their contents from degradation and is very stable. In contrast to nonhost vectors, exosomes are relatively nonimmunogenic and do not induce immune rejection. Exosomes easily cross biological barriers, especially blood-brain barriers. Organophilic factors, such as integrins on the surface of exosomes, can deliver drugs to specific tissues, thus improving the targeting specificity<sup>[12,79,80]</sup>. Because of these characteristics of exosomes, they are considered as ideal delivery carriers.

As a new drug delivery method, exosomes are used to deliver biomolecules and chemotherapy drugs in cancer therapy. MSCs can incorporate and deliver paclitaxel to recipient cells through exosomes, which have strong antitumor effects<sup>[81]</sup>. Exosomes act as nanoparticles to deliver anti-miR-214 and reverse the resistance of GC to cisplatin, which may become a potential alternative for cisplatin refractory GC treatment in the future<sup>[82]</sup>. Exosomes derived from macrophages can be used as carriers to deliver exogenous miR-21 inhibitors to GC cells, which can promote cell migration and inhibit cell apoptosis. Compared with traditional transfection methods, exosome-mediated miR-21 inhibitor delivery has a greater inhibitory effect and less cytotoxicity, demonstrating the potential of miR-21 and exosomes as therapeutic carriers for GC<sup>[63]</sup>.

### *Exosome vaccines*

Because exosomes can deliver tumor-derived antigens that activate CTLs, they are expected to be used for cancer immunotherapy. Tumor cell-derived exosomes may represent a new type of cancer vaccine. Exosomes from heat-treated malignant ascites can promote the maturation of DCs and induce tumorigenic CTL response, suggesting that heat stress can improve the immunogenicity of exosomes from malignant ascites in GC patients<sup>[83]</sup>. However, TDEs carry many oncogenes, which can induce tumor progression, so the safety of TDEs vaccine is still uncertain. DC vaccines can be quickly eliminated by antigen-specific CTLs. Since DC-derived exosomes express MHC-I and MHC-II molecules, which can promote the T-cell immune response and tumor rejection,



DC-derived exosome vaccines have a longer lifespan than DC vaccines, so they are considered as a substitute for DC vaccines<sup>[41]</sup>.

### *Cancer immunotherapy based on exosomes*

Exosomes have potential applications in cancer immunotherapy. The purpose of tumor immunotherapy is to establish durable and effective anticancer immunity. Tumor-specific CTLs play important roles in this process<sup>[84]</sup>. PD-L1 inhibits T-cell activation by binding to its receptor PD-1, thereby maintaining immune homeostasis. TDEs carry PD-L1 and thus resist immune checkpoint therapy. Exosomal PD-L1 may have the potential to become a target to overcome therapeutic resistance to anti-PD-1/PD-L1 antibodies<sup>[85]</sup>. Some studies have proposed that exosomes derived from CAR-T cells can replace CAR-T cells in anticancer immunity<sup>[86]</sup>.

### **EXOSOMES AND DRUG RESISTANCE IN GC**

Drug resistance of GC to conventional treatment is one of the factors causing its poor prognosis. In recent years, the following findings have illustrated the mechanisms of exosomes in the chemotherapy resistance of patients with GC<sup>[87]</sup>: (1) Exosomes can excrete cytotoxic drugs to mediate chemotherapy resistance; (2) Exosomes can deliver anti-apoptotic agents in cancer cells to interfere with drug metabolism, thereby promoting chemotherapy resistance; (3) Stromal exosomes induce drug resistance in cancer cells; and (4) Drug-sensitive cells become drug-resistant after treatment with exosomes from drug-resistant cells. The natural structure of exosomes makes them good carriers for chemotherapy drugs and good candidates for restoring therapeutic activity. Exosomes loaded with miRNA, mRNA, and other ncRNAs can mediate resistance. In recent years, the targeted delivery of miRNAs *in vitro* to alter the metabolism of receptor cells has become a rapidly developing new method.

### *Cancer cell-derived exosomes and therapeutic resistance*

Cisplatin is one of the most effective and commonly used basic chemotherapy drugs in the treatment of advanced GC. Exosomes from cisplatin-resistant GC cells deliver miR-500a-3p by targeting FBXW7 *in vitro* and *in vivo*, thus enhancing DDP resistance and stemness of GC cells<sup>[88]</sup>. However, some studies have found that exogenous anti-214 can reverse the resistance of GC cells to cisplatin and inhibit tumor growth<sup>[82]</sup>. miR-107 secreted by exosomes significantly enhances <sup>1</sup>the sensitivity of drug-resistant GC cells to chemotherapy drugs through the HMGA2/mTOR/P-gp pathway<sup>[89]</sup>.

#### *Microenvironment-derived exosomes and therapeutic resistance*

CAFs promote tumor progression and drug resistance by secreting various bioactive substances, including exosomes. MSCs have also been linked to drug resistance in GC. MSC-exosomes suppress 5-fluorouracil-induced apoptosis in GC cells, and enhance the expression of multidrug resistance-related proteins such as multidrug resistance-related protein, multiple drug resistance and lung resistance protein, thus inducing resistance of GC cells to 5-fluorouracil<sup>[90]</sup>. TAMs are the most abundant immune cells in the tumor microenvironment. Exosomal miR-21 derived from TAMs can be transferred directly to GC cells, inhibit apoptosis by downregulating PTEN, enhance the activation of PI3K/AKT signalling pathway, and promote cisplatin resistance in GC cells<sup>[91]</sup>.

#### **CONCLUSION**

Exosomes not only participate in the regulation of immunity, but also participate in various tumor processes, including growth, invasion, metastasis, angiogenesis and therapeutic resistance, and have value in disease diagnosis and prognosis evaluation. Due to the related biological properties, exosome-based GC therapy shows great promise. Using exosomes as carriers to deliver tumor suppressor molecules and drugs alone or in combination with traditional therapies, or blocking the release of GC cell-derived exosomes, all of these methods provide new strategies for the treatment of GC. Although exosomes are a relatively new area of research, in the field of cancer therapy, there has been widespread interest in their potential applications as cancer markers,

effective and safe anti-cancer drug delivery methods or as new inhibitors in immunotherapy.

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