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Roles of exosomes in cancer chemotherapy resistance, progression, metastasis and immunity, and their clinical applications (Review)

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Abstract. Exosomes are a type of vesicle that are secreted by cells, with a diameter of 40-100 nm, and that appear as a cystic shape under an electron microscope. Exosome cargo includes a variety of biologically active substances such as non-coding RNA, lipids and small molecule proteins. Exosomes can be taken up by neighboring cells upon secretion or by distant cells within the circulatory system, affecting gene expression of the recipient cells. The present review discusses the formation and secretion of exosomes, and how they can remodel the tumor microenvironment, enhancing cancer cell chemotherapy resistance and tumor progression. Exosome-mediated induction of tumor metastasis is also highlighted. More importantly, the review discusses the manner in which exosomes can change the metabolism of cancer cells and the immune system, which may help to devise novel therapeutic approaches for cancer treatment. With the development of nanotechnology, exosomes can also be used as biomarkers and for the delivery of chemical drugs, serving as a tool to diagnose and treat cancer.

Contents

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1. Introduction

In 1967, Peter Wolf first discovered that platelets can release numerous vesicles. At that time, researchers believed these vesicles were simply cell fragments with no relevant biological function (1). In 1983, exosomes were first observed in sheep reticulocytes (2). Johnstone named these vesicles 'exosomes' in 1987 and defined them as vesicles with a diameter of between 40 and 100 nm (3). In 2013, the Nobel Prize in physiology or medicine was awarded to James E. Rothman, Randy W. Schekman and Thomas C. Südhof for their study of extracellular vesicles (EVs) (4), which resulted in this evolving into a highly studied field.

Exosomes are EVs that originate from the endosome system and are important carriers of cell-to-cell communication in the microenvironment (5). Tumor cells can interact with immune cells, mesenchymal cells and endothelial cells in the microenvironment to promote tumor progression (6). Tumor exosomes can carry biological information in the form of proteins, lipids and nucleic acids. These molecules can domesticate recipient cells and may become tumor-specific markers (7). Therefore, exosomes and cells have significant interactions.

In clinical applications, circulating EVs derived from cancer patients are associated with tumor metastasis or recurrence; therefore, they can be used as important diagnostic and prognostic indicators, and therapeutic targets (8). In addition, exosomes, as natural nanoparticles, have great advantages over artificial materials in encapsulating chemotherapy drugs, such as paclitaxel (9). However, we do not have a complete understanding of EVs, which hinders the application of them as clinical treatments or diagnostic methods (10). Kalluri and LeBleu (11) stated that animal models with which biogenesis, trafficking and cellular entry of exosomes can be studied should be investigated rapidly. The study also summarized the challenges affecting the exosome field, such as improving the

exosome isolation method, the influence of the administration route for exosome uptake on potential therapeutic strategies, and combining exosomal RNA, protein and metabolites to enhance the specificity and sensitivity of exosome-based diagnostics.

2. Formation and secretion of exosomes

Cells capture extracellular materials via endocytosis and then form early endosomes (Fig. 1). During the process of transition to a late endosome, the endocytic body sprouts inside to envelop specific proteins and nucleic acids to form intraluminal vesicles (ILVs). Advanced endosomes containing multiple ILVs are known as multivesicular bodies (MVBs) (12). Most MVBs will be digested by lysosomes in the cells. Only a few vesicles containing cluster of differentiation (CD)63, recombinant lysosomal-associated membrane protein (LAMP)1 and LAMP2 on the surface can fuse with the cell membrane and mediate the secretion of exosomes (13). ILVs and MVBs act as pre-exosomes and are formed primarily with the assistance of endosomal sorting complexes required for transport (ESCRT) protein complexes, which are mainly composed of ESCRT-0, I, II and III, as well as auxiliary proteins vacuolar protein sorting-associated protein 4 (VPS4), vacuolar protein sorting-associated protein 1 (VTA1) and apoptosis-linked gene 2 interacting protein X (ALIX). The main function of ESCRT is to sort specific substances into ILVs. ESCRT-0 contains a hepatocyte growth factor-regulated tyrosine kinase substrate (HRS) that recognizes ubiquitinated proteins and interacts with signal-transducing adaptor molecules (14). HRS recruits ESCRT-I through tumor susceptibility gene 101 protein, and then ESCRT-I recruits ESCRT-III through ESCRT-II and ALIX (15). ESCRT-III forms exosomes by cutting MVBs (16), These exosomes are then released from the cells by fusion with the cell membrane. Rabphilin (RAB)27A/B, a member of the RAB family, is the main motion controller of exosomes on the cytoskeleton (17) Additionally, vesicle-associated membrane protein 7 of the Soluble N-etylmaleimide-sensitive fusion protein attachment protein receptor proteins family plays the main role in promoting the fusion between exosomes and cell membranes to secrete exosomes (18). Some studies have found that Dicer and Argonaute 2, the key components of miRNA processing, are functionally present in exosomes. Although it has yet to be confirmed, these findings suggest that exosomes are not only passive vehicles but also miRNA factories (19,20). Wang *et al* (21) reported that chemotherapeutic agents can stimulate the production or release of exosomes. Wei *et al* (22) found that pyruvate kinase M2, a key enzyme in aerobic glycolysis (known as the Warburg effect) of tumor cells, can promote the phosphorylation of synaptosomal-associated protein 23 and the subsequent release of exosomes. VPS33B is also a protein that regulates the release of exosomes, the deficiency of which will cause maturation and secretion disorder of exosomes (23).

3. Associations between exosomes and the tumor microenvironment (TME)

Exosomes were thought of as cellular metabolism waste products for a long time. With an increasing number of studies on exosomes, researchers have discovered that exosomes are important in cellular communication (24-26). Moreover, exosomes can affect tumor growth, angiogenesis, invasion and metastasis (27,28). Exosomes can promote the formation of the tumor microenvironment (TME) (29), which is comprised of tumor blood vessels, the extracellular matrix (ECM) and other non-malignant cells such as stromal cells, fibroblasts and inflammatory cells (30,31). Therefore, therapies that target the TME may be an effective treatment for cancer (32,33). Differing from that in normal cells, the metabolism of tumor cells relies on aerobic glycolysis even under normoxic conditions. This can generate more lactic acid and thereby lower the pH of the TME (34,35).

Exosomes can also influence the proliferation and differentiation of cells in the TME. Osteosarcoma-derived exosomes can promote osteoclast differentiation and bone resorption activity. In glioblastoma, exosomes derived from cancer cells that contain CD171 can promote glioma cell invasiveness, motility and proliferation (36). In breast cancer, MDA-MB-231 cell-derived exosomes can transfer miR-20a-5p to bone marrow macrophages and promote the proliferation and differentiation of osteoclasts by targeting SRC kinase signaling inhibitor 1 (37). Exosomes derived from mesenchymal stem cell (MSC)-differentiated adipocytes are actively taken up by breast cancer MCF7 cells. This subsequently promotes MCF7 cell proliferation and migration, as well as protecting the cells from serum deprivation or chemotherapeutic drug-induced apoptosis *in vitro* (38). Cancer cell-derived exosomes can promote differentiation of healthy fibroblasts or MSCs into cancer-associated fibroblasts (CAFs), which in turn activate cancer cell invasion and migration (39,40). Cancer cell loss of tumor protein p53 can result in adrenergic trans-differentiation of tumor-associated sensory nerves by EVs (41).

4. Exosomes can enhance tumor cell chemotherapy resistance

Cancer cell resistance to chemotherapeutics and monoclonal antibodies is one of the difficult issues affecting the successful treatment of patients. The mechanism behind this resistance is quite complicated (Table I).

In experimental studies on exosomes, it was found that drug-resistant tumor cells can transfer their drug resistance to drug-sensitive cells through exosomes. For example, the highly tumorigenic and drug-resistant cell subpopulations in glioblastoma multiforme (GBM) can regulate other subpopulations through microRNAs (miRNAs/miRs) in EVs (42). Long non-coding (lnc)RNA regulator of Akt signaling associated with hepatocellular carcinoma and renal cell carcinoma exosomes can competitively bind miR-34/miR-449 to increase the expression of lipoxin A4 receptor and cellular-mesenchymal epithelial transition factor in RCC cells, regulating the resistance of these cells to sunitinib (43). Vemurafenib can induce expression of miR-211-5p in melanoma cells, while cells overexpressing this miRNA will deliver miR-211-5p to other melanoma cells through exosomes, and thereby enhance drug resistance (44). Gemcitabine promotes the expression of miR-155 in exosomes secreted by pancreatic ductal adenocarcinoma (PDAC) cells and can deliver it to other PDAC cells to increase their drug resistance (45). Another study reported

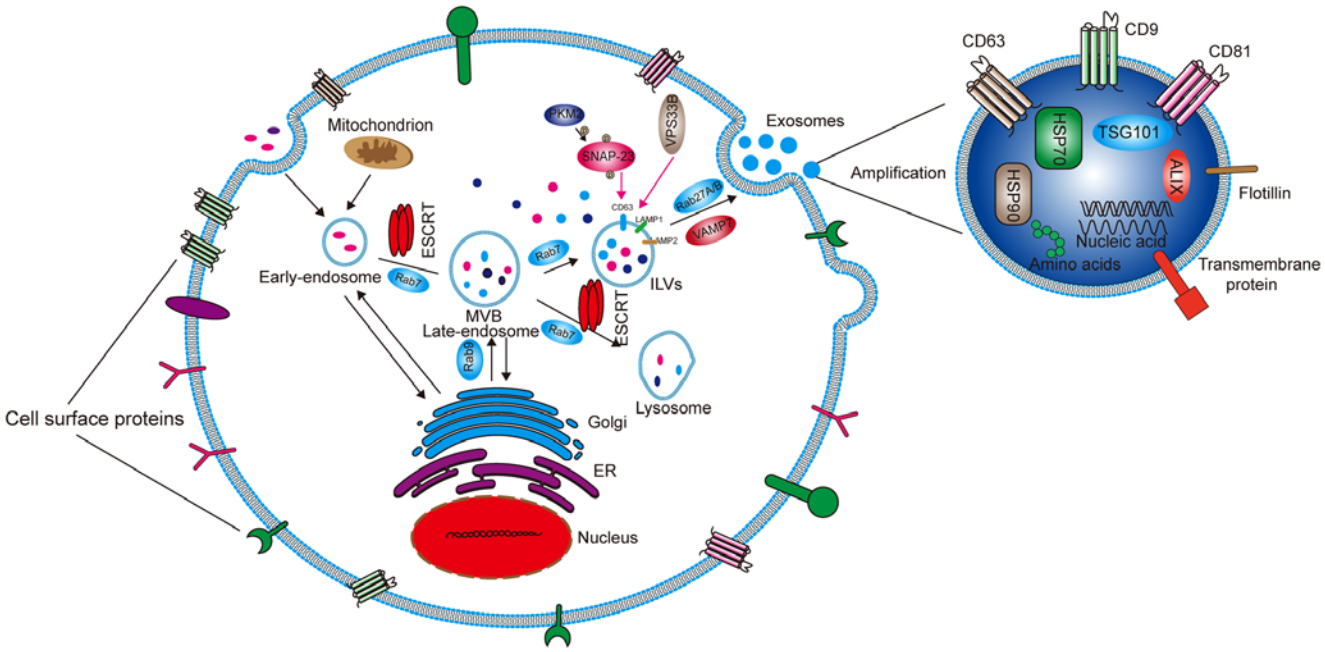

Figure 1. Formation and secretion mechanism of exosomes and the main components of exosomes. Early endosomes are formed through endocytosis, and then as a result of ESCRT, become late endosomes and then MVBs. After screening, some MVBs become ILVs and some are digested by lysosomes. ILVs are broken down to form exosomes and then released outside the cell. The whole process requires the action of Rab (an ATP protein) to provide ATP. The exosomes mainly contain HSP70, HSP90, TSG101, ALIX, amino acids and nucleic acids. The membrane surface mainly contains transmembrane proteins such as CD9, CD63, CD81 and Flotilin. ESCRT, endosomal sorting complexes required for transport; MVB, multivesicular body; ILV, intraluminal vesicle; Rab, rabphilin; HSP, heat shock protein; TSG101, tumor susceptibility gene 101 protein; ALIX, apoptosis-linked gene 2 interacting protein X; CD, cluster of differentiation; ER, endoplasmic reticulum; VAMP, vesicle-associated membrane protein; LAMP, recombinant lysosomal associated membrane protein; SNAP-23, synaptosomal-associated protein 23; VSP33B, vacuolar protein sorting-associated protein 33B; PKM2, pyruvate kinase M2.

that exosomes derived from ontogenically transformed, mesenchymal human bronchial epithelial cells (HBECs) could transfer chemoresistance to the parental, epithelial HBECs and increase zinc finger E-box binding homeobox 1 mRNA, a master epithelial-mesenchymal transition (EMT) transcription factor, in the recipient cells (46).

Moreover, exosomes secreted from stromal cells in the TME can also activate tumor-related pathways to enhance tumor cell resistance to drugs. The decreased sensitivity of breast cancer to anthracycline chemotherapy may be from exosomes secreted by peripheral cells promoting the expression of miR-125b in breast cancer cells (BCCs) (47). EVs from bone marrow stromal cells can enhance the resistance of chronic lymphocytic leukemia B cells to several chemotherapy drugs, such as fludarabine, ibrutinib, idelaisib and venetoclax (48). Exosomal miR-92a-3p derived from cancer-associated fibroblasts (CAFs) inhibits F-box/WD repeat-containing protein 7 and modulator of apoptosis 1 by activating the Wnt/β-catenin pathway, and ultimately inhibits mitochondria-related apoptosis (49). In PDAC, after the first-line gemcitabine treatment, release of CAF exosomes can increase the expression of epithelial chemotherapy resistance factor Snail and promote tumor resistance and proliferation (50). Physical factors will also affect the exosomes secreted by tumor cells. In a previous study, the phosphorylation of anaplastic lymphoma kinase (ALK) in exosomes released by the non-small lung cancer H3122 cell line after irradiation was enhanced, and H3122 cells treated with these exosomes showed significant resistance to ALK-specific inhibitors such as crizotinib, ceritinib and TAE684 (51).

Finally, EV-VEGF90K derived from BCCs can interact with heat shock protein (Hsp)90, blocking the antivascular effect of bevacizumab, which can be eliminated by Hsp inhibitors (52).

5. Role of exosomes in tumor progression

Exosomes act as a communication tool in the TME and promote tumor growth and invasion. Firstly, the exosomes produced by stromal cells in tumors have an impact on tumor cells. For example, Lazar *et al* (53) found that, in the presence of adipocyte exosomes, fatty acid oxidation is increased in melanoma cells, leading to increased migration and invasion. Secondly, exosomes secreted by tumor cells can also affect stromal cells to promote tumor cell metastasis and invasion. α-smooth muscle actin is a common biomarker of CAFs. Highly metastatic (HCC) cell-derived exosomal miR-1247-3p directly targets β-1, 4-galactosyltransferase III, resulting in activation of the β1-integrin-nuclear factor-κB signaling pathway in fibroblasts. Activated CAFs further promote cancer progression by secreting pro-inflammatory cytokines, including interleukin (IL)-6 and IL-8 (54). Additionally, exosomal miR-105 derived from BCCs repress the expression of MAX-interacting protein 1 (an antagonist of MYC transcriptional activity) in CAFs, thereby inducing its MYC activation-related gene expression and promoting tumor cell growth (55). Notably, McAtee *et al* (56) found that treating prostate stromal cells with tumor exosomes robustly stimulated their migration in a manner dependent on hyaluronidases 1 (Hyal1) catalytic activity, which explains

Table I. Intercellular communication through exosome-derived contents in basic research and research of therapeutic purposes.

System	Cancer type	Exosomal contents	Donor	Recipient	Target(s)	Function	Type	(Refs.)
Respiratory system	Lung cancer	ZEB1 mRNA	Mesenchymal NSCLC cells	Epithelial NSCLC cells	KRAS	Promotes epithelial NSCLC cell EMT progression	Drug resistance	(46)
		Phosphorylated ALK	H3122 treat with radiation	H3122	AKT/STAT3/ERK	The ALK protein cargo in exosomes could be a key element to drive tumor growth and compromise therapeutic efficacy of ALK inhibitors for ALK-positive NSCLC	Drug resistance	(51)
		miR-23a	NSCLC cells treat with hypoxia	Endothelial cells	PHD1/2	Increases vascular permeability and ability of tumor cell migration	Metastasis	(62)
	Nasopharyngeal cancer	miR-23a	Nasopharyngeal cancer cells	HUVECs	TSGA10	Promotes the growth, migration and microtubule formation of HUVECs	Metastasis	(59)
Digestive system	PDAC	miR-155	MiaPaCa2	Panc1	TP53INP1	High miR-155 expression is related to GEM resistance via an anti-apoptotic pathway	Drug resistance	(45)
	Hepatoma	14-3-3ζ	HCC	TILs	PD-1, TIM-3, LAG3 and CTLA-4	Decreases TIL antitumor activity	Immune inhibitor	(87)
		miR-1247-3p	HCC	Fibroblast	B4GALT3	Activates NF-κB pathway and promote tumor growth	Proliferation	(54)
		miR-103	HCC with high invasiveness	Endothelial cells	VE-Cad, p120, ZO-1	Increases vascular permeability and promotes cancer metastasis	Metastasis	(64)
		EGFR	Gastric cancer cell	Liver stromal cells	miR-26a/b	Activates hepatocyte growth factor	Metastasis	(68)
	Colorectal cancer	miR-92a-3p	CAFs	SW480/SW620/LoVo	FBXW7/MOAP1	Activates Wnt/β-catenin	Drug resistance	(97)
		miR-1246	Mutp53 colon cancer cells	Macrophages	hnRNPa2b1	Increases TAMs to secrete TGF-β	Proliferation	(96)
Reproductive system	Breast cancer	VEGF90K	MDAMB231	MDAMB231	VEGF	Causes bevacizumab to be ineffective in blocking MV-dependent VEGF receptor activation	Drug resistance	(52)
		miR-105	Breast cancer cells	CAFs	MXI1	Activates MYC pathway and promote tumor growth	Proliferation	(55)

Table I. Continued.

System	Cancer type	Exosomal contents	Donor	Recipient	Target(s)	Function	Type	(Refs.)
		miR-200c/miR-141	Metastatic breast cancer	Breast cancer cells	ZEB1/2, Wnt, Snail	Promotes cancer cell metastasis	Metastasis	(61)
		miR-126a	MDSCs treated with DOX	Th2 T cells and endothelial cells	S100A8/A9	Promotes tumor angiogenesis	Metastasis	(65)
		/	Metastatic breast cancer cells	T cells/NK cells	/	Inhibits T-cell proliferation and NK cell cytotoxicity	Immune inhibitor	(88)
	Prostate cancer (PC)	Hyal1	PC cells with Hyal1 overexpression	Fibroblasts/stromal cells	FAK	Increases mobility of fibroblasts and stromal cells	Metastasis	(56)
Others	Melanoma	miR-211-5p	MML-1 with vemurafenib treatment	MML-1	MITF	Transfection of miR-211 in melanoma cells reduces the sensitivity to vemurafenib treatment	Drug resistance	(44)
		FAO	Adipocytes	Melanoma cells	/	Increases the migration and invasion of melanoma cells	Progression	(53)
		snRNAs	Melanoma cells	Lung epithelial cells	TLR3	Promotes neutrophil recruitment and chemokine secretion	Metastasis	(68)
	Leukemia	/	BM-MSCs	CCL B cells	CCL3/4, EGR1/2/3, MYC	Increases CCL-B chemoresistance to several drugs, including fludarabine and ibrutinib	Drug resistance	(48)
	Liposarcoma	miR-25-3/ miR-92-3p	Liposarcoma cells	Macrophages	TLR7/8	Stimulates TAMs to secrete the IL-6	Immune inhibitor	(97)
		Integrin α2β1	SACC cells	CAFs	TGF-β	Enhances ECM remodeling in the lungs	Metastasis	(67)
		/	HNC patient serum	T cells	NKG2D	Promotes $CD8^+$ T-cell apoptosis, inhibit $CD4^+$ T-cell proliferation	Immune inhibitor	(86)

TIL, tumor-infiltrating T lymphocyte; NSCLC, non-small cell lung cancer; EMT, epithelial-mesenchymal transition; miR, microRNA; snRNA, small nuclear RNA; HUVEC, human umbilical vein endothelial cell; GEM, gemcitabine; DOX, doxorubicin; HCC, hepatocellular carcinoma; CAF, cancer-associated fibroblast; TAM, tumor-associated macrophage; MDSC, myeloid-derived suppressor cell; NK, natural killer; BM-MSC, bone marrow-mesenchymal stem cell; HNC, head and neck cancer; MV, micro vesicle; SACC, salivary adenoid cystic carcinoma; PDAC, pancreatic ductal adenocarcinoma.

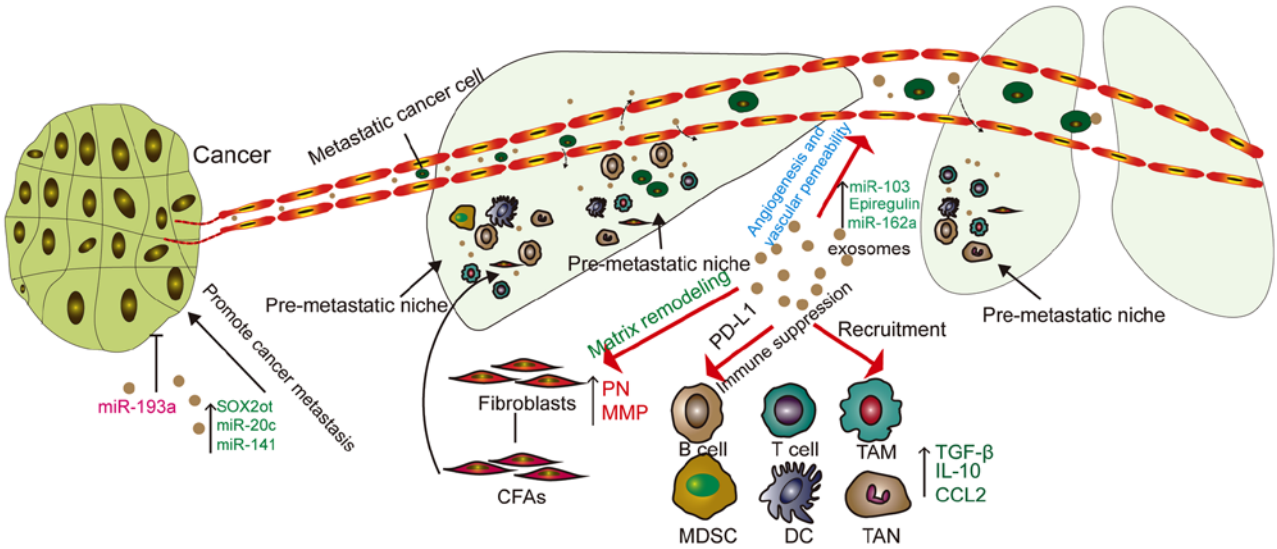

Figure 2. Functions of TDEs in cancer metastasis. TDEs can increase the permeability of blood vessels, which means that cancer cells are more likely to pass through blood vessels and seed in organs. TDEs can also recruit fibroblasts and immune cells, leading to matrix remodeling, causing upregulation of MMP and PN, forming a pre-metastatic niche and promoting tumor metastasis. TDE, tumor derived exosome; miR, microRNA; MMP, matrix metalloproteinase; SOX2ot, SRY-related high-mobility-group box protein-2 overlapping transcript; CAF, cancer-associated fibroblast; MDSC, myeloid-derived suppressor cell; DC, dendritic cell; TAM, tumor-associated macrophage; TAN, tumor associated neutrophil; CCL2, cell chemokine ligand 2; TGF-β, transforming growth factor β; IL, interleukin; PD-L1, programmed death ligand 1; PN, periostin.

why high levels of Hyal1 promote prostate cancer progression. In a study of the interaction between cancer cells and fibroblasts, Becker *et al* (57) found that tumor-derived EVs can cause differentiation of fibroblasts into myofibroblasts, which release matrix metalloproteinase (MMP) and remodel the ECM.

Exosomes secreted by tumor cells can change the characteristics of vascular endothelial cells and promote angiogenesis. For example, in the case of tumor cell hypoxia, MSC-derived EVs regulate the formation of blood vessels through various miRNAs, such as miR-126, miR-214 and miR-296 (58). miR-23a from nasopharyngeal carcinoma is rich in exosomes, which can promote the growth, migration and microtubule formation of human umbilical vein endothelial cells (59). In breast cancer, stromal fibroblasts can acquire the hallmarks of CAFs as a result of the loss of p85α expression. Exosomes derived from p85α-deficient fibroblasts can promote cancer progression via EMT induced by the canonical Wnt pathway (60).

6. Exosomes promote tumor metastasis

The cancer metastasis process involves several steps; it starts with the local infiltration of cancer cells, after which they enter the circulation through the lymphatic system or blood vessels. From the circulation, cancer cells need to enter and exit from the remote organs. Exosomes are involved in all steps of this process (Fig. 2).

Exosomes can increase the EMT effect of tumor cells. For example, in a previous study, the levels of miR-200c and miR-141 in the plasma of patients with metastatic breast cancer were significantly higher than those in the healthy group. Additionally, exosomal miR-200c and miR-141 derived from metastatic breast cancer also transferred to other breast cancer cells to promote their metastasis and EMT (61).

Lung cancer cells produce more exosomes under hypoxic conditions, and miR-23a is significantly upregulated in the exosomes from these cells. This leads to hypoxia-inducible factor-1α (HIF-1α) accumulation in endothelial cells, which promotes the formation and permeability of blood vessels, as well as tumor migration (62). Moreover, hypoxia also increases gastric cancer (GC) exosome release and miR-301α-3p expression in a HIF-1α-dependent manner, which promotes GC progression and metastasis (63). The expression level of miR-103 of exosomes in highly invasive HCC cells is significantly higher compared with that in non-metastatic HCC cells. Endothelial cells treated with highly invasive HCC cell-derived exosomes also show greater permeability (64). Unexpectedly, doxorubicin induced bone marrow stem cells to secrete exosomes containing IL-13R and miR-126a in breast cancer-bearing mice, which further induced $IL13^+$ T-helper (Th)2 cells to promote tumor angiogenesis (65).

Exosomes secreted by tumors can promote the formation of pre-metastatic niches (PMNs). EVs secreted by tumor cells form metastatic niches at remote sites of metastasis (57). The matrix environment of the niche prior to transfer is mainly composed of fibroblasts, endothelial cells and ECM. Fibroblasts not only induce inflammation and growth factors, but also express fibronectin and MMP (66). In a mouse model, CAF-derived EVs induced PMN formation in mouse lungs, increasing salivary adenoid cystic carcinoma lung metastasis (67). Exosomes in gastric cancer cells can be delivered to the liver and integrate on the plasma membrane of liver stromal cells. Exosomal epidermal growth factor receptor can inhibit the expression of miR-26a/b and activates liver cell growth factors, providing a supportive environment for gastric cancer cells (68). The study by Liu *et al* (69) demonstrated that lung epithelial cells are critical for initiating neutrophil recruitment and lung metastatic niche formation by sensing tumor exosomal RNAs via Toll-like receptor 3 (TLR3), providing suitable conditions for tumor metastasis.

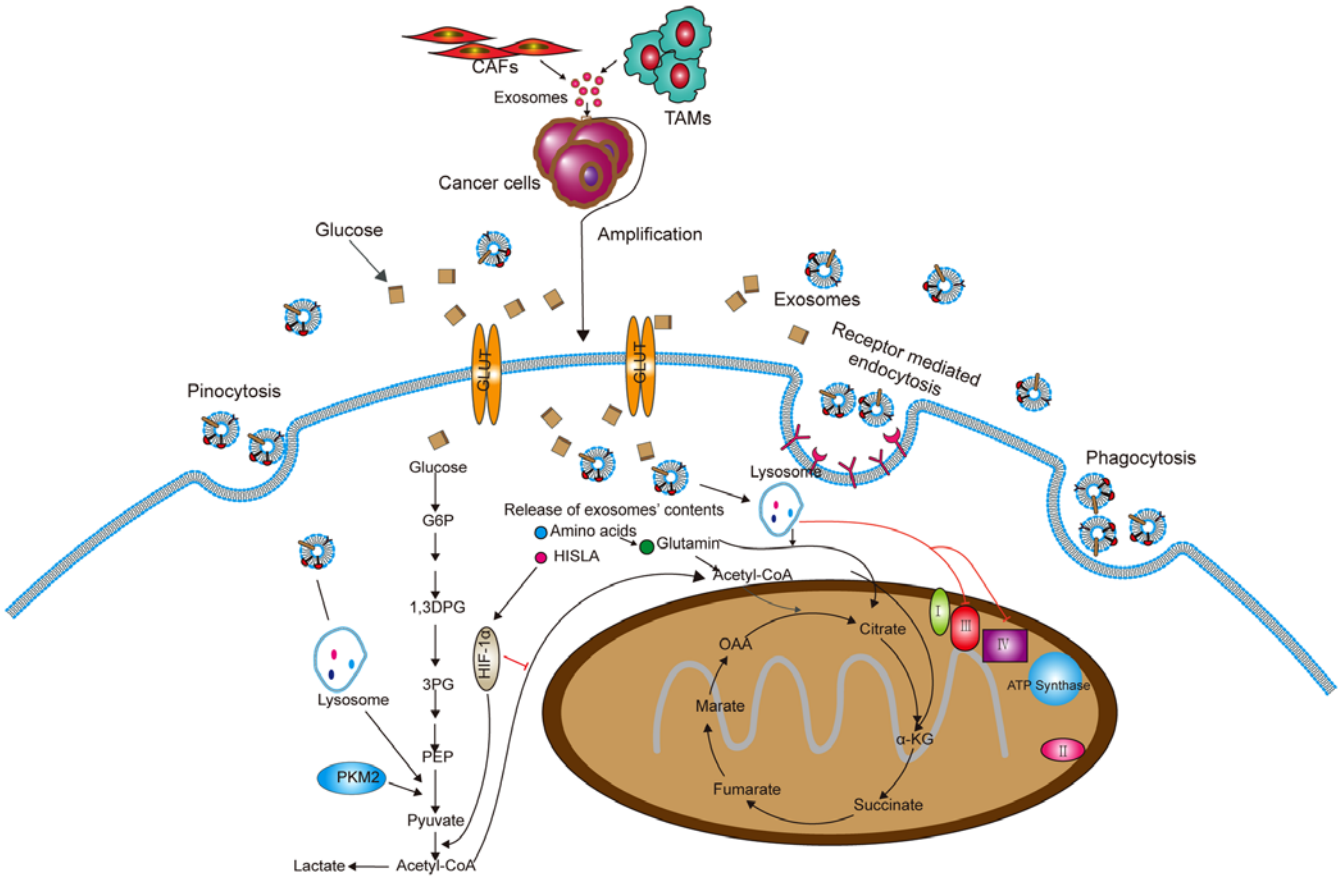

Figure 3. Exosomes derived from CAFs and TAMs can be taken up by cancer cells and change the metabolism in cancer cells. Exosomes secreted by non-tumor cells change the metabolism of tumor cells. Exosomes enter into cancer cells mainly by endocytosis, pinocytosis and receptor-mediated endocytosis. The amino acids and HISLA released from exosomes inhibit oxidative phosphorylation and cause glycolysis and glutamine-dependent reductive carboxylation. CAF, cancer-associated fibroblast; TAM, tumor-associated macrophage; HISLA, HIF-1α-stabilizing long non-coding RNA; PKM2, pyruvate kinase M2; PEP, phosphoenolpyruvate; 1,3DPG, 1,3-bisphosphoglyceric acid; G6P, glucose-6-phosphate; OOA, oxaloacetic acid; α-KG, α-ketoglutarate; GLUT, glucose transporter.

7. Exosomes can alter tumor cell metabolism

Metabolic reprogramming is an important characteristic of cancer cells, and this is usually caused by the TME (70). Cellular events related to the metabolic pathway include the Warburg effect, changes in Krebs cycle metabolites and the rate of oxidative phosphorylation, which may provide energy and structural requirements for the development and invasiveness of cancer cells (71) (Fig. 3). Researchers have found that exosomes secreted by CAFs in tumors can rebuild tumor metabolism. Specifically, CAF-derived exosomes could inhibit mitochondrial oxidative phosphorylation, thereby increasing glycolysis and glutamine-dependent reductive carboxylation (72). In breast cancer, the exosomes secreted by CAFs containing lncRNA small nucleolar RNA host gene 3 can sponge miR-330-5p, and miR-330-5p can target pyruvate kinase M1/M2 (PKM1/2). Therefore, exosomes from CAFs can positively regulate PKM expression, inhibit mitochondrial oxidative phosphorylation, increase glycolytic carboxylation and enhance breast tumor cell proliferation (73).

In addition, exosomes from tumor-associated macrophages (TAMs) contain HIF-1α-stabilizing lncRNA (HISLA). HISLA blocks the interaction of prolyl-4-hydroxylase domain-containing proteins 2 and HIF-1α to inhibit the hydroxylation and degradation of HIF-1α, thereby promoting cancer cell oxyglycolysis (74). These findings provide a new idea for targeting exosomes to treat tumors.

8. Effects of exosomes on the immune system

Exosomes not only affect the growth, invasion and metastasis of tumor cells, but can also modulate the immune system of the body. Moreover, exosomes can promote anti-tumor immunity and, in a number of ways, inhibit the immune system within the TME (75) (Fig. 4).

Exosomes in the antitumor immune response. Tumor-derived exosomes or EVs can activate immune responses. According to various reports, exosomes from tumor cells present neoantigens and/or major histocompatibility complex (MHC) peptide complexes, trigger and activate T cells through direct presentation and cross presentation through dendritic cells (DCs), or directly activate natural killer (NK) cells or macrophages (76-78). For example, HSP70-80 and MHC-I molecules of exosomes derived from tumor antigens and tumors can interact with DCs to induce effective $CD8^+$-dependent anti-tumor effects on mouse tumor T cells (79). In addition, studies have reported that tumor antigen proteins can be encapsulated

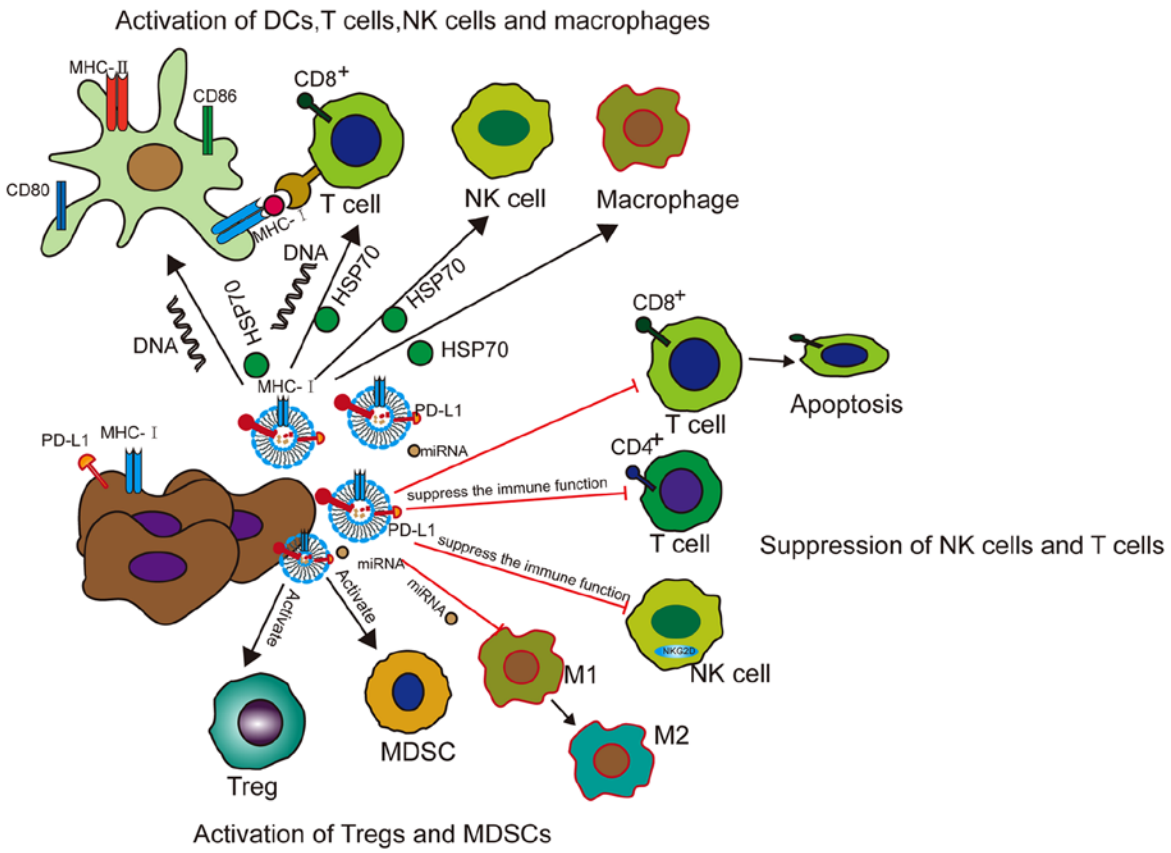

Figure 4. Functions of exosomes in tumor immunity. Tumor exosomes present new antigens with MHC-1 complex (such as HSP70) to DCs or directly activate T cells. Tumor exosomes increase the expression of CD80, CD86 and MHC-II in DCs, thereby further activating CD4+ T cells. Exosomal DNA triggers the activation of DCs and CD8+ T cells. Tumor exosomes induce the activation of NK cells and macrophages by transferring HSP70. DCs release exosomes containing antigen and MHC-1 complex to activate cytotoxic T cells to inhibit tumor growth. To promote the tumor immune response, tumor exosomes also inhibit the functions of DCs, T cells and NK cells, enhance the population of MDSCs and Tregs, and make macrophages. The function is biased towards the M2 phenotype. Tumor exosomes carry PD-L1 from tumor cells, transfer it to DCs or macrophages, and then block T-cell function. DC, dendritic cell; MHC, major histocompatibility complex; HSP70, heat shock protein 70; CD, cluster of differentiation; NK, natural killer; MDSC, myeloid-derived suppressor cell; Treg, regulatory T cell; PD-L1, programmed death ligand 1; miRNA, microRNA.

by vesicles secreted by DCs, drained to the lymph nodes and then transferred between subpopulations of DCs. Synapses are formed between interacting DCs. Vesicle metastasis occurs in the case of exosomes. Studies have shown that DCs containing vesicles can activate T cells, whereas DCs lacking vesicles cannot. These results help predict the immune response and provide new methods for immunotherapy (80-82).

Treating BCCs with topotecan, an antitumor drug that causes DNA double-strand breaks, can induce the release of exosomes. Consequently, exosomal DNA derived from cancer cells treated with topotecan can trigger DC activation and subsequent CD8+ T-cell activation through the cyclic GMP-AMP/stimulator of interferon genes signaling pathway (83).

Exosomes released from melanoma cells can be taken up by CD11b+ bone marrow (BM) cells, which can lead to the expansion and recruitment of patrolling monocytes and the differentiation of tumor necrosis factor-related apoptosis inducing ligand-positive tumor-reactive macrophages, and finally kill and phagocytose tumor cells (8).

The ability of exosomes to express antigen and MHC complexes, as well as induce helper T-cell immune responses, increases the possibility that exosomes can be used as anti-cancer vaccines. It is worth noting that HSP is an effective Th1 adjuvant. Heat stress can induce the expression of HSPs and MHC-1 in tumor cells, which can result in an increase in the immunogenicity of these cells. Exosomes prepared with heat-stressed carcinoembryonic antigen (CEA)-positive tumor cells can significantly induce cytotoxic T lymphocyte (CTL) responses, indicating that exosomes derived from heat-stressed tumor cells can be used as an effective vaccine for cancer immunotherapy (84).

Tumor-supportive immune reactions. Exosomes not only play a role in antitumor immunity, but they also inhibit the immune function in tumors. Unexpectedly, exosomes participate in all known mechanisms by which cancer can evade the immune system (85).

Firstly, exosomes can modulate regulatory T cells (Tregs). For example, exosomes in the plasma of patients with head and neck cancer (HNC) carry immunosuppressive molecules and interfere with the functions of immune cells via downregulation of NK cell group 2D expression in NK cells. Exosomes in the plasma of patients with active disease significantly induce the apoptosis of CD8+ T cells, suppress CD4+ T-cell proliferation and upregulate Treg suppressor functions to promote tumor growth (86). In a study on liver cancer cells, the content of 14-3-3ζ in exosomes secreted by these cells increased

significantly. This content increased after being taken up by tumor-infiltrating T lymphocytes (TILs). The TILs then showed significant immune activation ($CD69^+$), proliferation ($Ki\text{-}67^+$) and decreased antitumor activity (87). Moreover, EVs can recruit and activate Tregs and myeloid-derived suppressor cells (MDSCs), and can inhibit $CD8^+$ T-cell-mediated apoptosis of tumor cells (56). Wen *et al* (88) probed exosomes secreted by mouse metastatic BCCs and found that they largely accumulated in the mouse lungs, and suppressed the immune cell functions, inhibited T-cell proliferation and decreased NK cell toxicity.

Notably, Poggio *et al* (89) found that exosomal programmed death ligand 1 (PD-L1) from tumor cells will suppress T-cell activation in the draining lymph node, which can promote the growth of prostate cancer cells and reduce the efficacy of immunotherapy. This also confirmed that exosomal PD-L1 can promote the formation of PMNs. Glioblastoma is a local and systemic immunosuppressive tumor that can secrete inflammatory cytokines to initiate an immune checkpoint response. In addition, EVs released by glioblastoma stem cells express PD-L1 on their surface and play a key role in mediating anti-CD3 monoclonal antibodies to inhibit $CD4^+$ and $CD8^+$ T-cell activation and proliferation (90). In the oral/esophageal malignant lesion model induced by 4-nitroquinoline 1 oxide, PD-L1-carrying exosomes isolated from the supernatant of a mouse HNSCC cell line blocked $CD4^+$ and $CD8^+$ T-cell infiltration into the TME, thereby accelerating tumor progression (91).

Haderk *et al* found that tumor cells can modulate the surrounding macrophages to avoid being cleared by them. For example, tumor cells can secrete exosomes, transmit immunosuppressive signals to monocytes, suppress immunity and promote tumor growth (92). Moreover, exosomes secreted by tumors can also modulate macrophages to the M2 type and promote tumor progression. For example, under hypoxic conditions, exosomes derived from epithelial ovarian cancer cells can deliver miRNAs to induce M2 macrophage polarization (93). This has also been observed in pancreatic cancer (94). In a study of head and neck cancer, the EMT transcription factor Snail directly activated miR-21 transcription in tumor cells, thereby producing miR-21-rich tumor-derived exosomes. The exosomes containing miR-21 were phagocytosed by $CD14^+$ monocytes, which then inhibited the expression of M1 markers and increased the expression of M2 markers (95). In colon cancer, exosomes released by cancer cells with mutated p53, are taken up by surrounding macrophages, and miR-1246 can reprogram tumor-associated macrophages (TAMs) to weaken their anti-inflammatory immune function and increase secretion of tumor growth factor (TGF)-β, thereby promoting tumor growth (96). Unexpectedly, exosomal miR-25-3p and miR-92-3p from liposarcoma stimulate TAMs to secrete the pro-inflammatory factor IL-6 in a TLR7/8-dependent manner. However, these inflammatory responses can still pass through the surrounding environment and promote the proliferation, invasion and metastasis of liposarcoma cells (97).

Overall, exosomes have both immune activation and immune suppression functions in cancer. The effects of activating immunity mainly depend on the antigen presentation of exosomes, while the immunosuppressive effects of exosomes mainly depend on the ligands, proteins and miRNAs they carry, which inhibit the activity of cytotoxic T cells or increase immunosuppressive cells. Understanding the underlying mechanisms of these two functions can help lead to the development of exosomes as a novel method to treat cancer.

9. Exosomes act as cancer biomarkers

Early diagnosis of tumors has always been a key factor in tumor treatment. The clinical treatment of benign tumors generally has a good prognosis. As exosomes exist in various body fluids of the human body, including the saliva, urine and blood, they can possibly be used as biomarkers for disease diagnosis and prognosis (98) (Table II).

Increased levels of circulating EVs are found in some common liver diseases such as hepatitis and liver cancer (99). In lung cancer, tumor-derived exosomal miRNAs include adenocarcinoma-specific miR-181-5p, miR-30a-3p, miR-30e-3p and miR-361-5p, as well as squamous cell carcinoma-specific miR-10b-5p, miR-15b-5p and miR-320b. These miRNAs can be used as early diagnosis markers of lung cancer (100). The expression level of lncRNA-urothelial cancer-associated 1 (lncRNA-UCA1) in the exosomes from hypoxic bladder cancer cells was significantly increased, and the expression level of lncRNA-UCA1 in the serum exosomes of patients with bladder cancer was also significantly increased. This lncRNA can therefore be used as a diagnostic biomarker for bladder cancer (101). Prostate cancer is a common cancer in men. The gold standard for diagnosis is a biopsy, which can easily cause infection and bleeding. Evaluating the recurrence by assessing the prostate-specific antigen (PSA) content in the blood is insufficient. However, examination of prostate cancer using exosomes in urine is particularly simple and fast. Moreover, the exosomes secreted by the tumor increase based on the acidic environment of this cancer, so that patients can express CD81 and PSA nanovesicles at high levels (102). In one study, after the removal of the prostate tumor, the glucuronic acid, D-ribose-5-phosphate and isobutyryl-L-carnitine contents in urine EVs from patients were all 2-26 times lower compared with those prior to surgery (103). Although the treatment success rate has reached 80-90% in acute lymphoblastic leukemia (ALL), BM biopsy still causes great pain to patients. Johnson *et al* (104) found that exosomes secreted by ALL cells contain high levels of CD19 compared with normal cells, which can be used to diagnose ALL. Moreover, cancer risk factors can influence the synthesis and release of exosomes. Radiation can induce the release of exosome by activating p53 transcription, which can stimulate the expression of tumor suppressor activated pathway-6 (105). In a study conducted by Wu *et al* (106), it was found that smoking can induce the release of EVs, >90% of which are exosomes. When neurons are infected with Zika virus, expression of neutral sphingomyelinase-2, an important molecule that regulates the production and release of exosomes, is activated (107). Numerous cancer types usually show defects in the structure and number of centrosomes. Research reports have shown that the presence of excessive centrosomes can increase the secretion of exosomes, and that lysosomal dysfunction causes cells with extra centrosomes to secrete more exosomes in PDAC (108,109).

Exosomes can also be used as a prognostic indicator of treatment. In a prospective study of patients with esophageal

Table II. Exosomal contents used as diagnostic and prognostic markers for different cancer types.

System	Cancer type	Exosomal contents	Sample source	Change in regulation	Clinical outcome	(Refs.)
Respiratory system	Lung cancer	Glandular cancer: miR-181-5p, miR-30a-3p, miR-30e-3p, miR-361-5p Squamous cell carcinoma: miR-10b-5p, miR-15b-5p, miR-320b	Plasma of early-stage NSCLC patients	Upregulated	Early diagnosis	(100)
	Nasopharynx cancer	miR-24-3p	Nasopharyngeal carcinoma cell lines and patient sera (T-EXOs)	Upregulated	Poor prognosis	(113)
Digestive system	Gastric cancer	miR-196, miR-92, miR-1307	Malignant ascites from GC patients/mouse malignant ascites xenografts	Upregulated	Poor prognosis	(111)
	ESCC	G-NchiRNA	Saliva from nude mouse ESCC xenografts/ESCC patients	Upregulated	Therapeutic response, recurrence and early detection	(110)
Renal system	Bladder Cancer	lncRNA-UCA1	5637 cell culture medium/bladder cancer patient plasma	Upregulated	Diagnostic biomarker for bladder cancer	(101)
	PCa	CD81/PSA	PCa patients	Upregulated	Early diagnosis for prostate cancer	(102)
		Glucuronate, D-ribose 5-phosphate, isobutyryl-L-carnitine	Urinary EVs from the patients	Upregulated		(103)
Others	MM	miR-18a, miR-let-7b	Plasma from MM patients	Upregulated	Poor prognosis	(112)
	Osteosarcoma	miR-135b, miR-148a, miR-27a, miR-9	Plasma from patients with bad chemotherapy response	Upregulated	Poor response to chemotherapy	(116)
		miR-133a, miR-124, miR-199-3p, miR-385		Downregulated		

EV, extracellular vesicles; PCa, prostate cancer; GC, gastric cancer; NSCLC, non-small cell lung cancer; lncRNA, long non-coding RNA; ESCC, esophageal squamous cell carcinoma; MM, multiple myeloma.

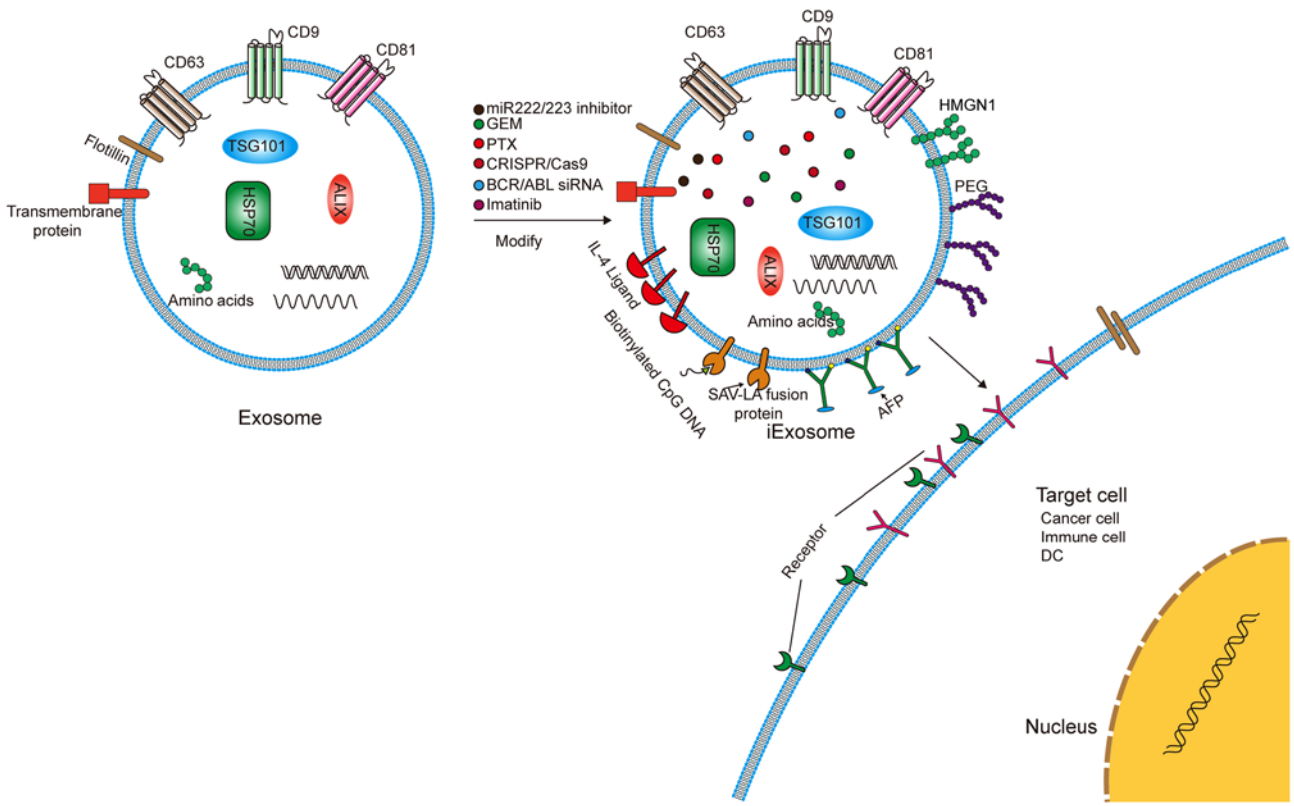

Figure 5. Exosomes can encapsulate chemical agents and be modified as engineered exosomes to deliver the agents to target cells. Exosomes can carry drugs such as GEM, PTX and imatinib and deliver them to recipient cells. Exosomes can also be loaded with the miRNA, siRNA or CRISPR/Cas9 system for tumor treatment. The surface of exosomes can be modified with SAV-LA or HMGN1 to increase the effect of antigen presentation. In addition, careful modification of the surface of exosomes with PEG or IL4 can increase the ability of the exosomes to avoid deletion and target tumor cells. GEM, gemcitabine; PTX, paclitaxel; CRISP/Cas9, clustered regularly-interspaced short palindromic repeats/CRISPR associated protein 9; BCR/ABL, breakpoint cluster region-c/abl oncogene 1; siRNA, short interfering RNA; miR, microRNA; SAV-LA, streptavidin-lactobacillus adhesion; HMGN1, high-mobility group nucleosome binding domain 1; PEG, polyethylene glycol; IL4, interleukin 4; DC, dendritic cell; HSP70, heat shock protein 70; CD, cluster of differentiation; ALIX, apoptosis-linked gene 2 interacting protein X; TSG101, tumor susceptibility gene 101 protein.

squamous cell carcinoma, the level of GOLM1-NAA35 chimeric RNA in the saliva of patients was used as a biomarker to evaluate treatment response, recurrence and early detection (110). Exosomes from the malignant ascites of patients with GC can promote the invasion of human gastric adenocarcinoma (AGS) cells. In mouse abdominal xenograft models using AGS cells, the addition of exosomes separated from malignant ascites decreased the median survival time of the mice significantly. Additionally, miR-196, miR-92 and miR-1307 are highly expressed in malignant ascites exosomes (111). Manier *et al* (112) showed that a cohort of 156 patients with newly diagnosed and uniformly treated multiple myeloma had exosomes containing miR-18a and miR-let-7b in the serum. Levels of these miRNAs were significantly associated with patient progression-free survival (PFS) and overall survival times. Exosomal miR-24-3p secreted by nasopharyngeal carcinoma cells can target fibroblast growth factor 11 to inhibit T-cell function. Studies have shown that patients with lower serum level of exosomal miR-24-3p have a longer PFS time (113-115). In osteosarcoma, Xu *et al* (116) found that in patients with OS and a poor chemotherapeutic response compared with in those with a good chemotherapeutic response, miR-133a, miR-124, miR-199-3p and miR-385 levels were significantly lower, while miR-135b, miR-148a, miR-27a and miR-9 were greatly overexpressed in serum exosomes. These data indicate that various exosomal RNA molecules may serve as reliable biomarkers for osteosarcoma tumors with different chemotherapeutic sensitivities.

10. Exosomes as a tumor treatment

Exosomes, as bioactive nanovesicle substances secreted by cells, can play an important role in targeted therapy (Fig. 5). The current clinical trials of exosomes in patients with cancer are summarized in Table III. Exosomes are stable, membrane-permeable, and can even pass through the blood-brain barrier. Additionally, exosomes can be combined with a number of physical materials and can therefore be used as natural nanoparticles for drug delivery and the delivery of miRNAs/small interfering RNAs (siRNAs) to treat diseases and tumors (117).

Exosomes have a double-layer membrane structure and nanometer size, which can protect them from clearance by the cell. This prolongs their circulating half-life and improves their biological activity (118). However, the half-life of an exosome in plasma is only 2-4 min (119). A variety of strategies have been proposed to improve the tumor cell targeting specificity and tumor uptake efficiency of exosomes. For example, the engineered exosomes, iExosomes, can target the Kirsten rat sarcoma viral oncogene protein for treating

Table III. Clinical trials of exosomes in patients with cancer.

System	Cancer type	Study	Type	Intervention	Status
Respiratory system	NSCLC	Trial of a vaccination with tumor antigen-loaded dendritic cell-derived exosomes	Interventional	Biological: Dex2	Phase I
		Study of exosome EML4-ALK fusion in NSCLC clinical diagnosis and dynamic monitoring	Observational	Drug: ALK inhibitor	Recruiting
	Lung cancer (diagnosis)	Serum exosomal long non-coding RNAs as potential biomarkers for lung cancer diagnosis	Observational	Diagnostic test: Collect samples	Recruiting
		Improving the early detection of lung cancer by combining exosomal analysis of hypoxia with standard of care imaging	Observational		Recruiting
	Pulmonary nodules	Clinical study of ctDNA and exosome combined detection to identify benign and malignant pulmonary nodules	Observational	Diagnostic test: ctDNA and exosome combined detection	Recruiting
Digestive system	Pancreatic cancer, benign pancreatic disease	Interrogation of exosome-mediated intercellular signaling in patients with pancreatic cancer	Observational	Blood, pancreatic fluid and pancreatic tissue	Recruiting
		Diagnostic accuracy of circulating tumor cells and onco-exosome quantification in the diagnosis of pancreatic cancer-PANC-CTC	Observational	Procedure: Blood samples. Procedure: Portal vein blood sample	Completed
		iExosomes in treating participants with metastatic pancreas cancer with KrasG12D mutation	Interventional	Drug: Mesenchymal stromal cell-derived exosomes with KRAS G12D siRNA	Phase I
	Pancreatic cancer	Circulating extracellular exosomal small RNA as potential biomarker for human pancreatic cancer	Interventional	Procedure: Venous sampling	Recruiting
	Colorectal cancer	Contents of circulating extracellular vesicles: Biomarkers in colorectal cancer patients	Observational	Biological: Analysis (protein, lipid, RNA) of circulating exosomes, size and number. Other: Gathering additional information about the cancer	Recruiting
	Rectal cancer	Exosomes in rectal cancer	Observational	Diagnostic Test: Blood Draw	Recruiting
Urinary system	Clear cell renal cell carcinoma	Evaluation of urinary exosomes presence from clear cell renal cell carcinoma	Observational	Biological: Urinary sample	Recruiting

Table III. Continued.

System	Cancer type	Study	Type	Intervention	Status
Reproductive system	Prostate cancer	Clinical validation of a urinary exosome gene signature in men presenting for suspicion of prostate cancer	Observational	Other: ExoIntelliScore prostate	Completed
		Exosomal microRNA in predicting the aggressiveness of prostate cancer in Chinese patients	Observational		Recruiting
		How does prostate cancer metastasize? Studying the role of secreted packages (exosomes) from fat tissue in lean and obese Patients	Observational	Procedure: Robotic Radical Prostatectomy	Recruiting
	HER2-positive breast cancer	A study to measure the expression of the HER2-HER3 dimer in tumour and blood (exosomes) samples from patients with HER2-positive breast cancer receiving HER2-targeted therapies	Observational	Other: Acquisition of blood samples and tumor tissue samples (biopsies)	Recruiting
Others	Oropharyngeal cancer	Exosome testing as a screening modality for human papillomavirus-positive oropharyngeal squamous cell carcinoma	Observational	None	Recruiting
	Head and neck cancer	Metformin hydrochloride in affecting cytokines and exosomes in patients with head and neck cancer	Interventional	Radiation: External beam radiation therapy. Drug: Metformin hydrochloride. Other: Placebo	Early phase I
	Sarcoma	Study of exosomes in monitoring patients with sarcoma (EXOSARC)	Observational	Biological: Blood samples	Recruiting
	Lung metastases osteosarcoma	Circulating exosome RNA in lung metastases of primary high-grade osteosarcoma	Observational	Diagnostic Test: ExoDx Prostate	Recruiting
	Lymphoma, B-cell, aggressive non-Hodgkin	Exosomes and immunotherapy in non-Hodgkin B-cell lymphomas	Interventional	Other: Blood sample	Recruiting

NSCLC, non-small cell lung cancer; siRNA, small interfering RNA; ctDNA, circulating tumor DNA.

pancreatic cancer (120). In lung cancer, exosomes modified with polyethylene glycol can improve the circulation time of paclitaxel-containing exosomes in the blood. Researchers have inserted RNA nanoparticles into the membrane of exosomes with tails that carry specific ligands. This enables the exosomes to target specific tumor cells and deliver the loaded siRNA into the cells (121). Notably, exosomes can also treat drug-resistant tumor cells. Imatinib can improve the prognosis of patients with CML; however, acquired resistance is still being encountered. Bellavia *et al* (122) attempted to transfer imatinib or breakpoint cluster region-c-abl oncogene 1 siRNA into exosomes fused with IL-3, which targeted CML cells and inhibited tumor growth *in vitro* and *in vivo*. Bliss *et al* (123) reported that BCCs can prime MSCs to release exosomes containing distinct miRNA contents, such as miR-222/223, which in turn promote quiescence and confer drug resistance in a subset of cancer cells. Building on these results, a novel, nontoxic therapeutic strategy was developed to target dormant BCCs based on systemic administration of MSCs loaded with antagomiR-222/223. In an immunodeficient mouse model of dormant breast cancer, this therapy sensitized BCCs to carboplatin-based therapy and increased host survival times. Cancer-derived exosomes function as natural carriers that can efficiently deliver clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR associated protein 9 plasmids to cancer cells, inhibit the expression of poly(ADP-ribose) polymerase family member 1 and induce the death of ovarian cancer cells (124). Milk-derived exosomes have been investigated for oral delivery of the chemotherapeutic drug paclitaxel as an alternative to conventional intravenous therapy for improved efficacy and reduced toxicity (125).

Another valuable application of exosomes is their use as anticancer vaccines. Exosomes secreted by M1 macrophages can promote an inflammatory response. Encapsulating the tyrosinase-related protein 2 vaccine with lipid calcium phosphate nanoparticle enhances its activity and induces a stronger antigen-specific cytotoxic T-cell response (126). In HCC, tumor-derived exosomes (TDEs) can elicit a stronger immune response than cell lysates *in vitro* and *in vivo* (127). Exosomes derived from α-fetoprotein (AFP)-expressing DCs (DEXAFP) and TDEs painted with the functional domain of high mobility group nucleosome-binding protein 1 elicited strong antigen-specific immune responses. Researchers found that HCC mice treated with DEXAFP had more γ-interferon (IFN-γ)-expressing $CD8^+$ T lymphocytes, high levels of IFN-γ and IL-2, fewer $CD25^+Foxp3^+$ Tregs, and lower levels of IL-10 and TGFβ in the tumors (128,129). Japanese researchers transfected B16BL6 cells with plasmids containing streptavidin (SAV), a high-affinity knot and biotin protein, and lactobacillus adhesion (LA), an exosomal promoting protein, to produce exosomes with expression of SAV-LA. This effectively enhanced the antigen presentation capabilities of the exosomes (130).

11. Conclusion and perspectives

Exosomes can contain biologically active substances such as proteins, nucleic acids and lipids. Exosomes can promote cancer cell drug resistance, invasion and metastasis. Therefore, the bioactive molecules contained within the exosomes can be used as a targeted therapy for treating tumors. In the clinic, the use of exosomes in body fluids to diagnose diseases and assess patient prognosis has also made significant progress. However, despite numerous studies and literature reports on exosomes, it remains a great challenge to translate these basic science studies to clinical applications. Most of the effects of exosomes on tumor progression or immunity have mainly been determined by 'gain-of-function' experiments, which may not fully represent the mechanism of action of exosomes in tumors. Secondly, as a biomarker for tumor diagnosis and prognosis, the extraction of exosomes is still a cumbersome task. How to extract them efficiently, rapidly and in large quantities remains a problem that needs to be addressed. Moreover, as a treatment method, the biological safety of exosomes still requires careful investigation. The next step is to decrease the cost of the extraction of exosomes, improve the safety of exosomes in clinical applications and develop a method to produce exosome-mimetic vesicles.

Although there are a number of challenges in their clinical application, exosomes are considered valid diagnostic biomarkers and potential therapeutic tools in cancer. Moreover, along with chemical, cellular and genetic engineering techniques, exosomal modification strategies may be promising for the development of clinical therapies.

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XW and YZ contributed equally to this study. KD is the correspondence author. XW was the main contributor in writing the manuscript. YZ was the main contributor in making tables and figures. KD was the main contributor in revising the manuscript critically for important content. All authors have read and approved the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

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Competing interests

The authors declare that they have no competing interests.

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