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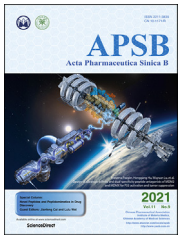

REVIEW

The biology, function, and applications of exosomes in cancer

Jinyi Liu[a,b], Liwen Ren[a,b], Sha Li[a,b], Wan Li[a,b], Xiangjin Zheng[a,b], Yihui Yang[a,b], Weiqi Fu[a,b], Jie Yi[c], Jinhua Wang[a,b,*], Guanhua Du[a,b,*]

[a]*The State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Beijing 100050, China*
[b]*Key Laboratory of Drug Target Research and Drug Screen, Institute of Materia Medica, Chinese Academy of Medical Science and Peking Union Medical College, Beijing 100050, China*
[c]*Department of Clinical Laboratory, Peking Union Medical College Hospital, Beijing 100730, China*

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KEYWORDS

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Abstract Exosomes are cell-derived nanovesicles with diameters from 30 to 150 nm, released upon fusion of multivesicular bodies with the cell surface. They can transport nucleic acids, proteins, and lipids for intercellular communication and activate signaling pathways in target cells. In cancers, exosomes may participate in growth and metastasis of tumors by regulating the immune response, blocking the epithelial–mesenchymal transition, and promoting angiogenesis. They are also involved in the development of

Abbreviations: ABCA3, ATP-binding cassette transporter A3; APCs, antigen-presenting cells; CAFs, cancer-associated fibroblasts; CCRCC, clear-cell renal cell carcinoma; CDH3, cadherin 3; CD-UPRT, cytosine deaminase-uracil phosphoribosyltransferase; circRNAs, circular RNAs; CRC, colorectal cancer; DC, dendritic cells; Del-1, developmental endothelial locus-1; DEXs, DC-derived exosomes; DLBCL, diffuse large B-cell lymphoma; DNM3, dynamin 3; ECM, extracellular matrix; EMT, epithelial–mesenchymal transition; ESCRT, endosomal sorting complex required for transport; GPC1, glypican-1; HA, hyaluronic acid; HCC, hepatocellular carcinoma; HIF1, hypoxia-inducible factor 1; hTERT, human telomerase reverse transcriptase; HTR, hormone therapy-resistant; HUVECs, human umbilical vein endothelial cells; ILVs, intraluminal vesicles; lamp2b, lysosome-associated membrane glycoprotein 2b; lncRNAs, long non-coding RNAs; MDSCs, myeloid-derived suppressor cells; MIF, migration inhibitory factor; miRNA, microRNA; MSC, mesenchymal stem cells; mtDNA, mitochondrial DNA; MVB, multivesicular body; ncRNA, non-coding RNAs; NKEXOs, natural killer cell-derived exosomes; NNs, nanoparticles; NSCLC, non-small cell lung cancer; PA, phosphatidic acid; PCC, pheochromocytoma; PDAC, pancreatic ductal adenocarcinoma; PD-L1, programmed cell death receptor ligand 1; PGL, paraganglioma; dsDNA, double stranded DNA; PI, phosphatidylinositol; PS, phosphatidylserine; PTRF, polymerase I and transcript release factor; RCC, renal cell carcinoma; SM, sphingomyelin; SNARE, soluble NSF-attachment protein receptor; TEX, tumor-derived exosomes; TSG101, tumor susceptibility gene 101.

*Corresponding authors. Fax: +86 10 63165313, +86 10 63165184.
E-mail addresses: wjh@imm.ac.cn (Jinhua Wang), dugh@imm.ac.cn (Guanhua Du).

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Biomarkers;
Drug delivery

resistance to chemotherapeutic drugs. Exosomes in liquid biopsies can be used as non-invasive biomarkers for early detection and diagnosis of cancers. Because of their amphipathic structure, exosomes are natural drug delivery vehicles for cancer therapy.

1. Overview of exosomes

Exosomes are subcellular vesicles averaging 100 nm in diameter enclosed by a lipid bilayer membrane. They have been found in nearly all body fluids, including blood, sweat, tears, urine, saliva, breast milk, ascites, and cerebrospinal fluid. Exosomes are part of novel intercellular communication system carrying and transmitting signal molecules that modulate the physiological state of cells and are closely related to the occurrence and progression of various diseases.

Exosomes were discovered in 1981 by EG Trams who found small vesicles surrounded by a bilayer membrane in supernatants of sheep reticulocytes cultured *in vitro*[1]. In 1987, Johnstone named these extracellular vesicles as exosomes[2]. Exosomes were originally considered to be garbage bins for ridding cells of degraded and unwanted cellular components. With continuing research progress on exosomes, it was gradually revealed that they were important for intercellular communication and involved in antigen presentation, cell differentiation, growth, the immune response in tumors, migration and invasion of tumor cells[3,4].

1.1. Composition and contents of exosomes

Exosomes carry various types of macromolecules from cells of different tissues and organs. The contents of exosomes are enwrapped within multivesicular body (MVB) membranes through inward invagination. The components of exosomes mainly include proteins, nucleic acids, and lipids (Fig. 1). Proteins commonly found in exosomes include those related to membrane transport, such as RAB GTPases, annexins, flotillins, MVB-producing proteins like ALIX, and tumor susceptibility gene 101 protein (TSG101). Exosomes also contain tetraspanins, including CD9, CD63, and CD81 and the heat shock proteins, HSP60 and HSP90[5–7]. Various nucleic acids were included in exosomes, such as microRNA (miRNA) and mRNA, the first two types to be identified[8,9], followed by lncRNA, tRNA, snRNA, snoRNA and circRNA[9,10]. These RNAs function as gene expression modulators and may be useful as potential biomarkers[11]. The lipid components in exosomes include sphingomyelin (SM), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidic acid (PA), ceramide, and cholesterol[12].

Exosome components are heterogeneous group, the composition of which reflects the cell type that produced them[4]. It was commonly believed that exosomes derived from the same type of cells contained similar proteins, nucleic acids, and lipids, but more recent studies revealed that the cargo depends not only on the cell type, but also on the origin of the cells. Proteomic profiling of breast cancer cells and their exosomes showed that exosomes secreted by epithelial and mesenchymal-like cells contained different proteins and nucleic acids[13]. The cholesterol and phospholipids contained in exosomes secreted by cancer cells and non-cancer cells are also different[14].

Currently, there are three databases to query the contents of exosomes, namely Vesiclepedia (http://microvesicles.org/index.html), ExoCarta (http://exocarta.org/index.html) and EVpedia (http://evpedia.info/)[15–17]. The three databases contained data of exosome contents of various organisms to help researchers explore the function of exosomes.

1.2. Biogenesis and release of exosomes

Exosome formation begins with endocytosis on the surface of the cell membrane with the formation of early endosomes *via* inward budding. Early endosomes mature into late endosomes, which encapsulate specific proteins, nucleic acids and other substances to form multiple intraluminal vesicles (ILVs), which are the precursors of exosomes. The late endosomes contain multiple ILVs and develop into MVBs[6,7,18]. Subsequently, most MVBs were fused with lysosomes, which resulted in degradation of container in MVBs. Membranes of a few MVB contain CD63, lysosomal membrane protein LAMP1, LAMP2, which can mediate their fusion with the plasma membrane and release exosomes to the extracellular milieu[6,19,20] (Fig. 2).

1.2.1. The mechanism of exosome biogenesis

The biogenesis of ILVs and MVBs is driven by a multiprotein complex called ‘endosomal sorting complex required for transport (ESCRT)’ that is composed of thirty kinds of proteins. The main function of ESCRT is to sort specific components into ILVs, which are the precursors of exosomes. ESCRT is made up of four kinds of complexes: ESCRT-0, ESCRT-I, ESCRT-II, and ESCRT-III, which contain specific proteins, VPS4, VTA1, and ALIX, etc. Each of the complexes plays a different role in the biogenesis of exosomes[6,19]. ESCRT-0 is responsible for cargo clustering in a ubiquitin-dependent process. ESCRT-I and ESCRT-II work synergistically to cause the endocytic membrane wrap specific molecules through germination. ESCRT-III drives scission of vesicles[19]. ESCRT-0 contains hepatocyte growth factor-regulated tyrosine kinase substrate (HRS), which can recognize ubiquitinated proteins and interact with STAM, another component of ESCRT-0. HRS can recruit TSG101 to ESCRT-I, and then ESCRT-I recruits ESCRT-III through ESCRT-II or ALIX[20].

Recent studies have shown that the biogenesis of exosome is reduced when the expression of HRS is inhibited in different cell types, such as HeLa cells, mouse dendritic cells (DC), HEK293 cells, and head and neck squamous cell carcinoma cells[21–24]. This indicates that HRS in ESCRT-0 has an important function in the biogenesis of exosomes. It was found that expression of the exosome markers, CD63 and MHC-II, was decreased, indicating inhibition of exosome release when STAM1 expression in ESCRT-0 and TSG101 expression in ESCRT-I were silenced by RNA interference in HeLa cells which have high expression of MHC-II[21]. The ESCRT−III−related protein, ALIX,

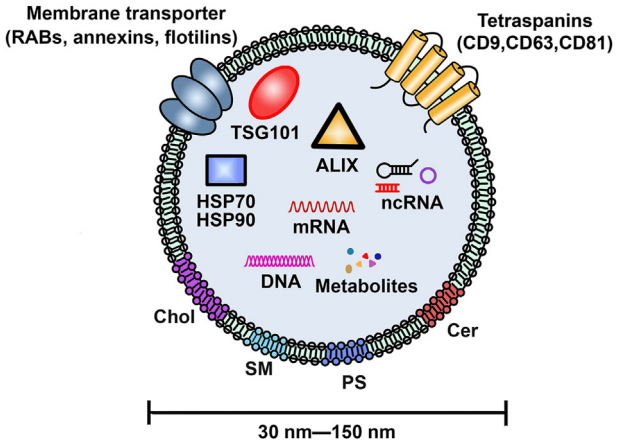

Figure 1 Schematic representation of structure of exosomes containing RNA, DNA, protein and metabolites surrounded by lipid bilayer.

can interact with syndecan−syntenin and also participate in the regulation of exosome formation. The expression of syndecan, ALIX and exosome markers, CD63 and HSP70, was increased in exosomes when syntenin was overexpressed whereas the expression of exosome syndecan, CD63 and HSP70 were decreased when ALIX or syntenin was knocked down in MCF-7 cells[25]. These results demonstrate that the ESCRT protein complex plays an important role in biogenesis of exosome.

ILVs and MVBs can also be generated by ESCRT-independent pathways involving lipids, tetraspanins, and HSPs. For example, the oligodendroglial precursor cell line, oli-neu cells, released exosomes with proteolipid protein when ESCRT was suppressed. Ceramide production was reduced when neutral sphingomyelinase was inhibited in oli-neu cells, which in turn inhibits the inward budding of MVB membranes and reduces release of exosomes[26]. Cholesterol is another lipid enriched in exosome membranes and an important component of MVBs[27]. Drug-induced or gene mutation-induced accumulation of cholesterol in MVBs can increase the secretion of flotillin-2, ALIX, CD63 and cholesterol-rich vesicles in a flotillin-2-dependent manner in oli-neu cells[28]. The membranes of MVBs and exosomes also contain tetraspanins[29], which are thought to be involved in sorting of exosome contents. The tetraspanin, CD63, can sort melanoma-related proteins and ceramide in melanoma cells into ILVs without ESCRT[30]. The heat-shock cognate protein, HSC70, can recruit transferrin receptor to exosomes[31]. Tumor cells can activate multiple signaling pathways to promote biogenesis of exosomes under hypoxic condition[32].

1.2.2. The mechanism of exosomes secreted from cells

After MVBs are produced in the cells, some degraded MVBs are fused with lysosome, which resulted in the degradation of MVBs and their contents. The secreted MVBs are fused with the plasma membrane and release exosomes to the extracellular environment. In recent years, the process of exosome secretion to the extracellular milieu has gradually been elucidated, which mainly relied on the assistance of the RAB family and the soluble NSF-attachment protein receptor (SNARE) family.

RABs are small GTPase proteins that interact with the cytoskeleton to control intracellular vesicle sprouting, transporting, and positioning on the plasma membrane[33], which suggests that RABs might be involved in secretion of exosomes. RAB11 was the first RAB protein reported to be associated with exosome secretion. RAB11 was related to the release of exosomes containing TFR and HSC70 from the myelogenous leukemia cell line, K562[34]. In oli-neu cells, inhibition of RAB35 reduced the secretion of exosomes loaded with proteolipid protein[35]. ShRNA-based screening results in HeLa cells showed that silencing RAB2B, RAB5A, RAB9A, RAB27A and RAB27B reduced the release of exosomes containing CD63, CD81, and MHC-II[36].

SNARE is a complex of multiple proteins, which can cause the fusion of two plasma membranes in contact with each other, and is thought to promote the fusion of vesicle membranes and plasma membranes[37]. VAMP7 is a member of the SNARE family. In K562 cells, VAMP7 is an essential component of exosomes containing acetylcholinesterase[38]. Inhibition of VAMP7 expression

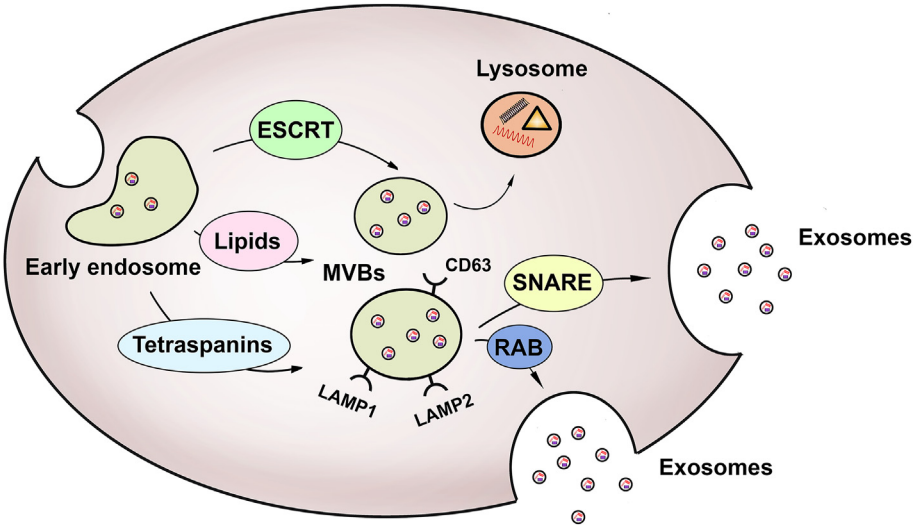

Figure 2 Schematic representation of the origin and release of exosomes. Exosomes are formed as ILVs by budding into early endosomes and MVBs. ESCRT, lipids and tetraspanins are involved in the biogenesis of MVBs. MVBs can be either fused with lysosomes or with the plasma membrane, which allows the release of their contents to the extracellular milieu. RAB and SNARE are involved in the transport of MVBs to the plasma membrane and secretion of exosomes.

prevented the release of secreted lysosomes and inhibited the secretion of exosomes to the extracellular environment in MDCK cells[39]. In HEK293 cells, the SNARE protein component YKT6 promoted the production of exosomes loaded with WNT3A morphogen[23].

1.3. Physiological functions of exosomes

Exosomes were originally thought to be extra-membranal proteins secreted by reticulocytes to regulate membrane function during maturation[2]. In 1996, Raposo et al.[40] found that some immune cells like B-lymphocytes could release antigen-presenting vesicles. The secreted exosomes directly stimulated the anticancer activity of effector $CD4^+$ T cells. In 2007, Valadi et al.[8] discovered that cells were able to exchange genetic material through RNA in exosomes. With these findings, scientists developed a better understanding of the composition and function of exosomes, and began to see how they might be used in diagnosis and treatment of disease. Exosomes are widely involved in material transport and signal transmission through the release of the specific components they carry into the recipient cells[4]. In pancreatic cancer cells, the *KRAS* mutation promotes macrocytosis and the absorption of exosomes[41]. Melanoma cells absorb exosome components by promoting the fusion of the plasma membrane with the exosomes[42]. *In vivo* studies in mice have shown that some exosomes can directly deliver mRNA to recipient cells, especially under the stimulation of acute or chronic infections[42]. Exosomes also have immunoregulatory activities including antigen presentation and immune tolerance. Exosomes carrying MHC class II complexes that bind to tumor-specific antigens were able to significantly inhibit tumor growth in mice[43]. These exosomes may indirectly activate naïve T cells and B cells by interacting with antigen-presenting cells, and may also promote the proliferation of $CD4^+$ T cells[44]. Exosomes derived from melanoma cells were found to carry programmed cell death receptor ligand 1(PD-L1), which could directly inhibit the anticancer function of $CD8^+$ T cells *in vivo*[45]. Tumor-derived exosomes (TEXs) inhibited the maturation of DCs in a PD-L1-dependent manner and thereby reduced the T cell-mediated immune response[46]. The nucleic acids carried by exosomes may regulate innate and adaptive immune activities, and exosomes derived from mesenchymal stem cells and tumor cells also played a special role in the immune response[4]. Exosomes were involved in the transmission of some viruses[47]. Exosomes promoted tumorigenesis and metastasis, and were associated with the acquisition of cancer resistance[4,48]. Thus, the unique structure and function of exosomes may allow them to be used as diagnostic biomarkers and their capacity for carrying a payload of proteins or nucleic acids to target cells may prove highly effective in developing novel cancer therapies that are less devastating to the body than chemotherapy.

2. The functional roles and mechanisms of exosomes in cancer

2.1. Exosomes and cancer immunity

Many studies have demonstrated a close relationship between exosomes and the development and progression of cancers[49,50]. In the tumor microenvironment, exosomes can transfer bioactive molecules between tumor cells, immune cells and stromal cells to help cancer cells escape immune surveillance and induce immune tolerance. In contrast, exosomes derived from immune cells can inhibit tumor growth, proliferation, and metastasis. Thus, exosomes play a double-edged sword role in cancer immunity.

2.1.1. Exosome-mediated cancer immunosuppression

Multiple studies have suggested that TEXs can promote immunosuppression[51,52]. TEXs have ligands on their surfaces that can bind to homologous receptors in immune cells and transmit inhibitory signals to them[53]. Exosomal PD-L1 is capable of mediating immune escape for cancer cells. If TEXs carrying PD-L1 enter the lymphatic system from the blood, they could inhibit the activity of T cells and prevent immune cells from recognizing and killing tumor cells[45,54]. Exosomes can also exert an immunosuppressive effect by inhibiting proliferation and inducing apoptosis of T cells by transmitting death signals through the FAS/FASL pathway[55]. Tumor exosomes were also able to inhibit NK cell activity and interfere with monocyte differentiation[52]. HSP72 carried by TEXs induced the STAT3-dependent immunosuppressive function of myeloid-derived suppressor cells (MDSCs), thereby achieving immune tolerance[56]. Besides, TEXs could prevent tumor cells from attacking by immune system and assist tumor cells to escape immunosurveillance[57]. The inhibition of type I interferon production by EGFR delivered by TEXs to host macrophages revealed a potential mechanism for the decline of cancer patient immunity[58].

2.1.2. Exosomes and cancer immunotherapy

The main goal of cancer immunotherapy is to stimulate the immunosuppressed host to recognize and eradicate cancer cells. Exosomes can be used as a delivery system for immunotherapy drugs, antigens or genes by modifying the cells that secrete exosomes[59]. Both immature and mature dendritic cells can produce exosomes, and DC-derived exosomes (DEXs) were able to overcome tumor-induced immunosuppression through direct and indirect means. The exosomes released by mature DCs contained higher levels of MHC I, MHC II and costimulatory molecules than those of immature DCs, which should endow them with stronger immune stimulating effects[60]. DEXs had MHC/peptide complexes on the surface, which promoted the proliferation and activation of IL-15Rα and NKG2D-dependent NK cells and enhanced T cell-dependent antitumor activity[61]. DEXs could also activate T cells, enhance T cell-mediated immune responses, and inhibit the growth of breast cancer cells[62]. DEX-based antitumor vaccines have entered phase I and II clinical trials after animal model testing[63,64]. Study showed that exosomes had no secondary toxicity at the maximum tolerated dose in fifteen patients with metastatic melanoma, indicating the safety of DEXs as tumor vaccine[65]. In the treatment of patients with non-small cell lung cancer, DEXs could carry the melanoma antigen gene MAGE, which demonstrated the feasibility and potential effectiveness of the DEX vaccine[66]. In addition, exosomes derived from mouse cell lines expressing hMUC1 promoted the immune response and antitumor activity against the growth of tumor cells expressing hMUC1 *in vivo*[59]. CAR-T cell-derived exosomes could replace CAR-T cells as an approach of tumor immunotherapy[67]. The exosomes secreted by antigen-presenting cells (APCs) were able to stimulate the proliferation of T cells *in vitro* and induce antitumor immune responses *in vivo*. TEXs contain tumor-associated antigens, costimulatory molecules, and MHC I and MHC II molecules, which may induce effective antitumor immune responses. TEXs can be cross-presented by APC to cytotoxic T lymphocytes, thus exerting tumor-killing effects[68]. Other study[54] has shown that inhibiting the release of PD-L1 from exosomes

may result in long-term, systemic tumor growth inhibition. This may constitute a valuable new immunotherapy direction in cancer treatment.

2.2. *Exosomes and cancer metastasis*

Tumor metastasis is the main cause of cancer death. For a long time, the research on the mechanism of tumor metastasis was focused on the interaction between tumor and body. However, in recent years, it has been discovered that exosomes play an important role in the metastasis of tumors by transmitting specific information between cells. Exosomes may also participate in regulation of the epithelial−mesenchymal transition (EMT) and extracellular matrix (ECM) remodeling. In addition, exosomes can promote tumor angiogenesis and immune escape, facilitate formation of pre-metastatic niches and determine organotropic metastasis (Fig. 3).

2.2.1. *Regulation of EMT*

The EMT was involved in a loss of epithelial cell polarity, extracellular matrix remodeling and the formation of pre-metastatic niches, which can enhance the ability of cancer cells to enter the circulatory system to promote metastasis of tumor cells. During the EMT process, epithelial tumor cells acquire mesenchymal characteristics under the influence of cancer-associated fibroblasts (CAFs) in the tumor matrix. The tumor cells lose polarity and cell−cell connections, then enter a low proliferation state with enhanced migration and invasion ability[69]. Many studies have shown that exosomes play an important role in EMT. Co-culture of nasopharyngeal cancer cell lines with exosomes isolated from similar cell lines increased expression of the mesenchymal marker proteins vimentin and N-cadherin and down-regulation of E-cadherin, indicating that exosomes could induce the EMT of nasopharyngeal carcinoma cells and promote tumor metastasis[70]. Treatment of urothelial cells with exosomes isolated from muscle-invasive bladder cancer cells produced a phenotype resembling the EMT and increased cell migration and invasion. In contrast to control exosomes from embryonic kidney cells, exosomes from the bladder cancer induced the expression of vimentin, snail and twist proteins in the urothelial cells, and reduced the expression of E-cadherin *via* the TGF-β1 pathway[71,72]. TEXs not only enable other tumor cells to acquire the mesenchymal phenotype, but also transform the mesenchymal stem cells into CAFs. TGF-β-positive TEXs increase the expression of α-SMA and transform stem cells into CAFs, which can stimulate EMT by secreting TGF-β1 and activating the TGF-β1-SMAD signaling pathway[73]. CAFs also produce exosomes, which can activate the WNT signaling pathway in breast cancer cells and enhance cell migration[74].

Oncogenic microRNAs (miRNAs) contained in exosomes are also involved in the regulation of EMT. MiR-23a in exosomes regulates the expression of E-cadherin in lung cancer cells and perpetuates the EMT process through the TGF-β pathway[75]. Exosomes from bone marrow-derived mesenchymal stem cells contain miR-193a-3p, miR-210-3p, and miR-5100, which can activate the STAT3 signaling pathway, induce EMT, and promote the invasion of lung cancer cells[76]. Exosomes derived from $CD103^+$ cancer stem cells promote metastasis of clear-cell renal cell carcinoma (CCRCC) cells by delivering miR-19b-3p and initiating EMT. CD103 can guide cancer stem cells-derived exosomes to target cancer cells and organs and confer greater lung metastatic capacity on CCRCC, suggesting that $CD103^+$ exosomes are potential diagnostic biomarkers for tumor metastasis[77]. MiR-21 in exosomes in the serum of patients with esophageal cancer was correlated with recurrence and metastasis of cancer, and was also a biomarker of tumor recurrence in lung cancer and melanoma[78−80].

2.2.2. *Regulation of tumor angiogenesis*

Angiogenesis is an essential process for growth and metastasis of tumors, especially under hypoxic conditions. Exosomes are important messengers between tumor cells and vascular endothelial cells in hypoxia-driven pro-angiogenic tumor responses[81]. Carbonic anhydrase 9 (CA9) is induced by hypoxia-inducible factor 1 (HIF1) in response to hypoxia and is overexpressed in renal cell carcinoma (RCC). It has been shown that CA9 in exosomes released from hypoxic RCC can enhance angiogenesis in the microenvironment, thereby promoting cancer progression[82]. In

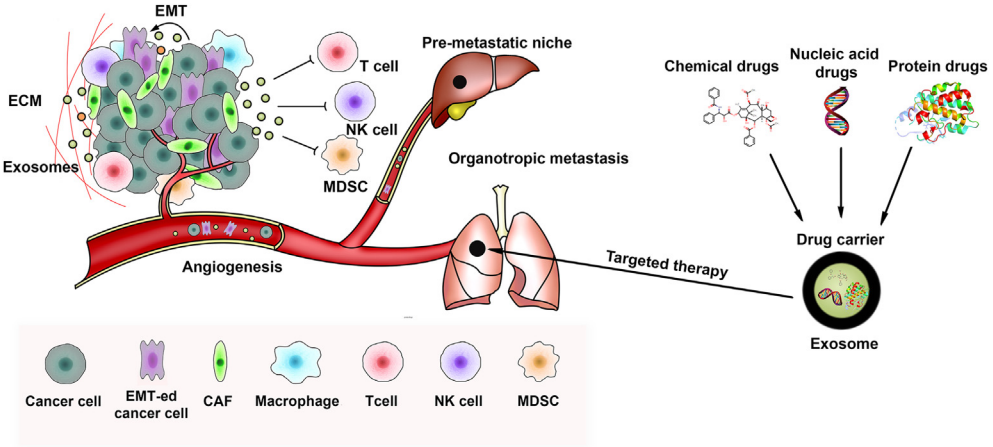

Figure 3 Schematic representation of exosomes in tumor metastasis and targeted therapy. Exosomes promote epithelial−mesenchymal transition, angiogenesis and extracellular matrix remodeling in the tumor microenvironment. Exosomes can also help tumor cells escape from immune surveillance, promote formation of pre-metastatic niches and allow cancer cells to invade and colonize distant organs. Different types of therapeutic payloads including small molecule drugs, proteins and nucleic acids can be loaded into exosomes, which can then be targeted to tumor tissues.

contrast to exosomes isolated from normal thyroid follicular cells, Nthy-ori-3-1, and normoxic papillary thyroid carcinoma cells, BCPAP, exosomes isolated from hypoxic BCPAP cells significantly enhanced angiogenesis in human umbilical vein endothelial cells (HUVECs). Under hypoxia, miR-21-5p was significantly upregulated in exosomes secreted by BCPAP cells, and directly targeted and inhibited TGF-β1 and COL4A1 to promote the formation of blood vessels. The angiogenic effect of exosomes isolated from hypoxic BCPAP cells with knockdown of miR-21-5p was inhibited, indicating that hypoxic papillary thyroid carcinoma cells increase the angiogenic pathway through exosomal miR-21-5p/TGF-β1 and miR-21-5p/COL4A1 interaction[83]. Exosomes secreted from melanoma cells can induce a pro-angiogenic signaling cascade characterized by vascular endothelial formation in a dose-dependent manner[84]. Exosomes secreted by head and neck squamous cell carcinoma can promote angiogenesis by reprogramming endothelial cells[85]. Exosomes released from mesenchymal stem cells (MSC) promote angiogenesis by transferring pro-angiogenic miRNAs (*e.g.*, miR-30b) to endothelial cells[86]. miR-100 are enriched in MSC-derived exosomes, which can promote angiogenesis in breast cancer by activation of the mTOR/HIF-1α signaling axis and decrease the expression of VEGF in breast cancer cells[87].

2.2.3. *Regulation of ECM remodeling*

The main components of the ECM include collagens, fibronectins, glycosaminoglycans, and proteoglycans, which are associated with alteration of the phenotype and function of tumor cells and stromal cells[88]. Exosomes can change the tumor microenvironment by mediating ECM remodeling and ultimately promote tumor metastasis. Exosomes derived from highly metastatic hepatocellular carcinoma (HCC) cells are loaded with oncoproteins, such as Met, caveolin and S100. After uptake by normal hepatocytes (MIHA cells), exosomes from the HCC activate the PI3K/AKT and MAPK signaling pathways and induce the secretion of active MMP2 and MMP9, promote degradation of ECM, and accelerate tumor cell migration and invasion[89]. MMP13 can degrade components of the ECM and exosomes loaded with MMP13 promote metastasis of nasopharyngeal carcinoma by degrading ECM[70]. Exosomes derived from melanoma contain integrin β1, which activates pro-MMP2 and degrades type one collagen and gelatin[90].

2.2.4. *Regulation of organotropic metastasis*

The target organ of tumor metastasis is not a random choice. In 1889, Paget[91] proposed the 'seed and soil' hypothesis, which is, certain tumor cells (seeds) tend to metastasize to specific organs (soils). Only when the soil is suitable for the seeds will they grow and successfully metastasize. Up to now, organ-specificity has continued as one of the biggest mysteries of tumor metastasis. Studies have shown that 'seeds' can affect the 'soil' by secreting exosomes before they arrive, thereby preparing the pre-metastatic microenvironment for metastasis of tumor cells. Proteomic analysis revealed that exosomes derived from tumor cells of different organs have different integrin expression profiles. Integrin $\alpha6\beta4$ and $\alpha6\beta1$ are related to lung metastasis, while integrin $\alpha v\beta5$ is related to liver metastasis. Knockdown of integrin $\alpha6\beta4$ and $\alpha v\beta5$ expression can reduce the acquisition of exosomes by target organ cells, which in turn reduces lung and liver metastases, respectively[92]. Studies of a pancreatic ductal adenocarcinoma (PDAC) mouse model showed that TEXs played an important part in promoting the implantation of metastatic pancreatic cells in the liver by interacting with Kupffer cells. Macrophage migration inhibitory factor (MIF) was highly expressed in PDAC-derived exosomes, and the inhibition of MIF expression prevented the formation of a pre-metastatic niche in the liver. Compared to patients without progression of pancreatic tumors, there was higher MIF expression in exosomes from patients with stage I PDAC who developed liver metastases. This finding indicated that MIF in exosomes could trigger liver metastasis and may be a prognostic biomarker for the liver metastasis of PDAC[93]. It was found in malignant melanoma that exosomes tended to colonize the interstitial spaces of kidney, liver, bone marrow and lung, and these organs were the sites where melanoma was prone to metastasis[94]. There was evidence that RNA from TEXs recruited neutrophils by activating alveolar epithelial TLR3 and promoted the formation of a pre-metastasis niche. There was less lung metastasis in the spontaneous metastasis model of TLR3-deficient mice[95].

The proliferation and metastasis of tumor cells is also related to their ability to escape from immune surveillance, and exosomes are instrumental in tumor immune escape (see Section 2.1.1). Thus, exosomes are involved in multiple processes of tumor metastasis to ensure the formation of the pre-metastasis niche and subsequent tumor metastasis and have the potential to be used clinically to predict and prevent tumor metastasis.

2.3. *Exosomes and tumor drug resistance*

Drug resistance of tumors is a common cause of therapeutic failure. In recent years, more and more studies have shown that exosomes can promote drug resistance through various mechanisms. Exosomes can transport miRNAs, lncRNAs and proteins to the target cells and achieve signal transmission between drug-resistant cells and sensitive cells, stromal cells and tumor cells, which can induce drug resistance of tumor cells[96–98]. The expression of miRNAs was different in exosomes secreted by MDA-MB-231 breast cancer cells compared to drug-resistant cells, indicating that these differential miRNAs may induce drug resistance[99]. Exosomes secreted by chemotherapy-resistant MCF-7 cells contain miR-221/222, which can make sensitive MCF-7 cells tamoxifen-resistance by inhibiting the expression of p27 and estrogen receptor α (ERα)[100]. In the microenvironment of ovarian cancers, exosomes secreted by tumor-associated adipocytes and tumor-associated fibroblasts can transfer miR-21 to ovarian cells, directly downregulate APAF1 expression, and inhibit tumor apoptosis, resulting in resistance to paclitaxel[101]. The EMT is also involved in regulating resistance of tumor. In MCF-7 and MDA-MB-231 cells, exosomal miR-155 targets TGF-β, FOXO-3a and C/EBP-β to induce tumor drug resistance by up-regulating the EMT markers SNAIL, SOX9 and EZH2[102]. In colorectal cancer (CRC), CAF exosomes loaded with miR-92a-3p target FBXW7 and MOAP1 in the tumor microenvironment to activate the WNT/β-catenin pathway, inhibit mitochondrial apoptosis, promote cell stemness, EMT, tumor metastasis, and 5-FU/L-OHP resistance[103].

The delivery of lncRNA in exosomes can also promote tumor resistance, as evidenced by increased expression of lncRNA H19 in a gefitinib-resistant human non-small cell lung cancer (NSCLC) cell line. lncRNA H19 can be encapsulated into exosomes specifically mediated by hnRNPA2B1 and transferred to non-drug-resistant NSCLC cells to produce gefitinib resistance[104]. Temozolomide-resistant glioblastoma cells can also remodel the

tumor microenvironment by secreting exosomes loaded with lncRNA SBF2-AS1 to promote chemotherapy resistance[105].

Proteins in exosomes can also affect tumor drug resistance. Exosomes containing ubiquitin C-terminal hydrolase-1 (UCH-L1) and P-glycoprotein (P-gp) proteins derived from MCF-7 breast cancer cells resistant to doxorubicin were secreted into the extracellular microenvironment and taken up by doxorubicin-sensitive MCF-7 cells, thereby leading to drug resistance[106]. One of the first-line drugs of prostate cancer, docetaxel, can induce hormone resistance in tumor cells. The MDR-1/P-gp multidrug-resistance efflux transporter carried in TEXs can enhance drug resistance in prostate cancer cells[107]. During treatment of $HER2^+$ breast cancer with trastuzumab (HER2 antibody), the cancer cells can secrete exosomes with high levels of HER2. Trastuzumab in the tumor microenvironment can bind to exosomal HER2 thus reducing its activity on tumor cells[57]. In addition, $HER2^+$ breast cancer cells can also secrete exosomes containing the immunosuppressive cytokine TGF-β1 and PD-L1, that increase resistance to trastuzumab[48]. Many studies were done in recent years to find ways to circumvent this exosome-mediated induction of drug resistance. The biosynthesis of exosomes is dependent on ATP-binding cassette transporter A3 (ABCA3) and inhibition of ABCA3 expression can increase drug retention in tumor cells. Diffuse large B-cell lymphoma (DLBCL) cells can acquire drug resistance by exosomes coated with adriamycin and picoxenone, and indomethacin can improve the anticancer effect of these drugs on DLBCL by inhibiting ABCA3[108]. In imatinib-resistant chronic myeloid leukemia, dasatinib can be used to inhibit the release of exosomes, downregulate AKT/mTOR activity, and reduce drug resistance of tumor cells[109].

3. Exosomes and cancer diagnosis

Identification of the contents in exosomes provides more information on specific biomarkers than traditional tumor biomarker assays. Monitoring tumor exosome contents for specific biomarkers would be a convenient non-invasive method for diagnosis and a way to follow disease progression and treatment.

3.1. Exosomal nucleic acids

Nucleic acids, such as mRNA, miRNA, lncRNA, circRNA and DNA, have all been found in exosomes. The mRNAs in tumor cell exosomes carries a large amount of genetic information, which can be used to diagnose diseases, and monitor treatment responses. It has been reported that the level of PD-L1 mRNA expression in plasma exosomes is related to the immunotherapy response in terms of PD-L1 antibodies in melanoma and non-small cell lung cancer. This study confirmed that mRNA in exosomes can be used as a biomarker for tumor immunotherapy and provide guidance for clinical treatment[110]. The mRNAs in urine exosomes of prostate cancer patients have the same transcriptome as in the prostate cancer cells. For example, the expression of cadherin 3 (CDH3) mRNA was downregulated in both PC tissues and corresponding patients' urine exosomes[111]. In another study, the human telomerase reverse transcriptase (hTERT) expression in exosomes from the plasma of pancreatic cancer and lung cancer patients was significantly higher than that of the control group, indicating that mRNA in exosomes can be useful for the early diagnosis of tumors[112]. The expression of dynamin 3 (DNM3) and P65 mRNAs in exosomes was also found to be upregulated in glioblastoma multiforme, which can be used for the diagnosis and treatment of this disease[113].

MiRNAs are small non-coding RNAs of about 22 nucleotides. The content of miRNAs is the highest among all types of RNA in exosomes. MiRNAs are sequestered from RNases within the exosome, which makes them more stable. MiRNAs in exosomes appear to be more useful as diagnostic tumor biomarkers than miRNAs in body fluids. Exosomal miRNAs are not randomly loaded into exosomes, but are recruited through a specific sorting mechanism[114]. The lipid membrane of exosomes prevents degradation of the contained miRNAs, allowing them to be maintained at higher concentration than intracellular miRNAs[115]. In 2008, Taylor et al.[116] first proposed diagnosing ovarian cancer by detecting specific miRNAs in serum exosomes, and further used miRNA tracking to predict the prognosis of patients. They found that the miRNAs in TEXs isolated from serum were closed associated with the miRNAs in ovarian tissue, and that there were significant differences between the miRNA level of the patient group and the control group. MiR-375 and miR-1307 in exosomes isolated from serum could be used as potential biomarkers in diagnosis of ovarian cancer and combined with traditional biomarkers CA125 and HE4, can improve the specificity and accuracy of cancer diagnoses[117]. The markers, miR-373, miR-200a, miR-200b and miR-200c, in exosomes were also associated with diagnosis and clinical prognosis of ovarian cancer[118]. Another study showed that expression of miRNA-34a, miRNA-34b and miRNA-34c were significantly downregulated in patients with hepatoblastoma, and that miRNA-34 could be used as a biomarker for diagnosis and prognosis of hepatoblastoma[119]. In hepatocellular cancer, the expression of miR-18a, miR-221, miR-222 and miR-224 in serum exosomes was significantly increased whereas expression of miR-101, miR-106b, miR-122 and miR-195 was significantly decreased, which suggests that miRNAs in serum exosomes could be used as biomarkers for diagnosis of HCC[120]. In breast cancer patients, miR-373, miR-21, miR-182, miR-1246 and miR-105 in exosomes could be used as promising serum biomarkers[121]. It has been reported that the miRNAs, let-7a, miR-1224-5p, miR-1229, miR-1246, miR-150, miR-21, miR- 223 and miR-23a, were significantly upregulated in exosomes from serum of patients with primary colon cancer and downregulated after surgery by detection with miRNA microarray assays[122,123]. MiR-320d in serum exosomes could be used to distinguish metastatic colorectal cancers from non-metastatic cancers[124]. The expression of exosomal miRNA-21 in cerebrospinal fluid was correlated with poor prognosis and recurrence of patients with glioma[125]. MiR-1290 and miR-375 in plasma exosomes were shown to be promising biomarkers in patients with castration-resistant prostate cancer[126]. In addition, the level of miR-2909 in urine exosomes could be used as a diagnostic marker of prostate cancer[127]. There are significant differences of miR-20a-5p, miR-103a-3p and miR-4505 in serum exosomes between multiple myeloma or smoldering myeloma patients and healthy individuals; thus, miRNAs in serum exosomes could be used as novel biomarkers of myeloma[128]. MiR-150, miR-1246 and miR-155 in serum exosomes could be used as early non-invasive biomarkers of acute myeloid leukemia[129] (Table 1[116–128]).

Long non-coding RNAs (lncRNAs) are a large group of RNAs longer than 200 nucleotides without the function of encoding protein due to that lack of a complete open reading frame. Exosomal lncRNAs could also be used as tumor biomarkers. HOTTP in serum exosomes is a potential biomarker for diagnosis of gastric cancers and has higher accuracy than traditional

biomarkers, such as CEA and CA19-9[130]. The expression of lncRNA-p21 in urine exosomes of prostate cancer patients was up-regulated compared with prostate hyperplasia patients; therefore, lncRNA-p21 could be used as a diagnostic marker of different stages of prostate cancer, which could better guide individualized therapy[131]. Upregulation of lncRNA PCAT-1 and MALAT1 in urine exosomes was correlated with poor recurrence-free survival, and could be used for diagnosis and recurrence prediction of bladder cancer[132]. LncRNA PTENP1 in exosomes secreted from normal cells could be transported into bladder cancer cells to inhibit progression of the disease[133]. Lee et al.[134] demonstrated that in hepatocellular carcinoma, the overall survival and progression-free survival were significantly lower in patients with higher circulating levels of exosomal lncRNA-ATB, thus lncRNA-ATB may be a novel diagnostic biomarker and therapeutic target.

Circular RNAs (circRNAs) are a group of endogenous non-coding RNAs (ncRNAs) consisting of a covalently closed circular molecule formed by reversed splicing. CircRNAs are highly conserved and stable, which are good characteristics of potential biomarkers for cancer diagnosis. Pan et al.[135] demonstrated that, compared with healthy individuals, expression of hsa-circ-0004771 in serum exosomes was increased in patients with CRC and could be a prospective biomarker for diagnosis of CRC. CircRNA PDE8A in TEXs can promote invasion of pancreatic cancers *via* the miR-338/MACC1/MET pathway, which could be a biomarker for diagnosing and monitoring progression of pancreatic cancers[136]. The level of circRNA PRMT5 in serum and urine in patients with urothelial carcinoma was increased and could be used to predict risk of metastasis[137]. In addition to the foregoing types of RNAs, tRNA, piRNA and snoRNA are also found in exosomes[138].

Genomic DNA and mitochondrial DNA (mtDNA) are also found in exosomes. Exosomal DNA is largely similar to the DNA in tumor cells from which the exosomes were released, which means that the DNA in exosomes can be used for detecting carcinogenic mutations[4]. The exosomes of patients with pheochromocytoma (PCC) and paraganglioma (PGL) contain double stranded DNA (dsDNA) that can reflect the mutation status of susceptible genes over almost all chromosomes. Exosomal dsDNA can be used as a non-invasive biomarker for genetic diagnosis and preoperative evaluation of PCCs and PGLs, and can be one of the most effective methods for somatic mutation screening[139]. The circulating exosomes in patients with hormone therapy-resistant (HTR) breast cancer can package and transfer of mtDNA and act as a carcinogenic signal to regulate the HTR breast cancer escape from dormancy[140]. However, some researchers do not believe that exosomes are carriers of extracellular DNA. A study[141] from Vanderbilt University showed that dsDNA and DNA-binding histones did not exist in exosomes or other types of extracellular vesicles. It is thought that extracellular dsDNA and histones are actively secreted through autophagy and MVE-dependent mechanisms, rather than *via* exosomes.

3.2. *Proteins in exosomes*

In recent years, with the popularization of proteomics technology, the composition and function of proteins in exosomes have been revealed and their roles in predicting tumor occurrence, diagnosis and development have received much attention. Many studies have shown that proteins loaded into exosomes are differentially expressed in different tumors. Melo et al.[142] analyzed numerous serum samples of patients with pancreatic cancer and found that the proportion of glypican-1 (GPC1)-positive exosomes was significantly increased compared with healthy people. Further study showed that the level of exosomal GPC1 in the serum of patients with early pancreatic cancer was significantly higher than that in normal people, and could be used to diagnose early and late pancreatic cancer with 100% accuracy and sensitivity. Serum exosomes from NSCLC patients contained overexpressed proteins, such as NY-ESO-1, EGFR, PLAP, EpCam and Alix, which are closely associated with poor overall survival[143]. In another study, 144 highly phosphorylated proteins were identified from the plasma exosomes of breast cancer patients using proteomics methods. Among this group of phosphoproteins, RALGAPA2, PKG1, and TJP2 had even higher phosphorylation level in breast cancer patients. Thus, it was believed that phosphoproteins in exosomes isolated from blood could be used as new biomarkers for the diagnosis and prognosis of breast cancer[144]. In addition, the expression of the ECM protein, developmental endothelial locus-1 (Del-1), in exosomes can help to identify breast cancer at an early stage and distinguish malignant breast cancer from benign hyperplasia and non-cancerous disease[145]. It was also found that polymerase I and transcript release factor (PTRF) in serum exosomes of glioma patients positively correlated with tumor grade, and could be used as a biomarker for tumor diagnosis as well as a potential therapeutic target[146]. Sun et al.[147] systematically compared the proteomes of salivary and serum exosomes of healthy volunteers and lung cancer patients by liquid chromatography/mass spectrometry and identified

Table 1 Exosomal miRNAs as biomarkers for diagnosis of cancers.

Cancer	miRNA	Biofluid	Ref.
Ovarian cancer	miR-373, miR-375, miR-1307, miR-200a, miR-200b, miR-200c	Serum	116,117
Hepatocarcinoma	miRNA-34, miR-18a, miR-221, miR-222, miR-224, miR-101, miR-106b, miR-122, miR-195	Serum	118,119
Breast cancer	miR-373, miR-21, miR-182, miR-1246, miR-105	Serum	120
Colorectal cancer	let-7a, miR-1224-5p, miR-1229, miR-1246, miR-150, miR-21, miR- 223, miR-23a, miR-320d	Serum	121−123
Glioma	miR-21	Cerebrospinal fluids	124
Prostate cancer	miR-1290, miR-375 miR-2909	Plasma Urinary	125,126
Multiple myeloma	miR-20a-5p, miR-103a-3p, miR-4505	Serum	127
Acute myeloid leukemia	miR-150, miR-1246, miR-155	Serum	128

differentially expressed proteins. This discovery of potential biomarkers provided a basis for the diagnosis of lung cancer.

3.3. *Exosomal lipids and small molecule metabolites*

Compared with source cells, sphingomyelin, phosphatidylserine, phosphatidylic acid, ceramide and cholesterol are generally enriched in exosomes[12]. Lipids in exosomes are necessary to maintain external morphology, but they can also be used as signal molecules for biological processes in tumors[12]. Studies have shown that lipids in exosomal membranes may transfer 'mobile rafts', thus converting exosomes into extracellular signaling bodies and spreading the activation of cell signaling pathways in tumorigenesis and metastasis. Ceramide may regulate the functions of 'mobile rafts' and their effects on signal pathways in the cell[148]. Skotland et al.[149] identified several different lipids in exosomes isolated from urine and cell culture supernatants from patients with prostate cancer and healthy controls by high-throughput mass spectrometry and quantitative lipidomics, and further confirmed the diagnostic value of exosomal lipids in prostate cancers. Using targeted ultra-high performance liquid chromatography with tandem mass spectrometry, Puhka et al.[150] analyzed one hundred metabolites in urine samples, platelets and exosomes isolated from patients with prostate cancer before and after prostatectomy. The results showed that the small-molecule metabolites in exosomes were associated with tumorigenesis. In addition, targeted quantitative lipidomics methods based on LC−MRM-MS were used to screen for potential candidate biomarkers in serum exosomes from patients with pancreatic cancer. The results revealed an imbalance of lipids in exosomes, which may be used as biomarkers for diagnosis of pancreatic cancer and help to uncover the pathological processes of pancreatic cancer[151].

With the current sophisticated analytical methods, the possibility of combining exosomal protein, lipid, RNA, and miRNA in cancer diagnosis and prognostic evaluation is currently being considered. Using a combination of exosomal biomarkers including metabolites, RNAs, and proteins that distinctively reflect aspects of disease could potentially improve the specificity and sensitivity of exosome-based cancer diagnosis[4]. Cho et al.[152] developed a method for multiplexed *in situ* detection of exosomal miRNAs and proteins, which allowed the quantitative analysis of various disease-specific miRNAs and surface proteins in prostate cancer cell-derived exosomes in a single reaction.

4. Exosomes as drug carriers for antitumor therapy

For most drugs, only a relatively small amount reaches the lesion to exert a therapeutic effect. This reduces the efficacy and can cause toxicity and adverse side effects to the patient. Exosomes, as natural carrier of intercellular information, participate in the exchange of biomolecules between cells, and therefore have great potential as novel drug carriers. Exosomes have many advantages, such as small size, natural molecular transport properties, and good biocompatibility[153]. Therefore, they are more suitable for use as drug delivery systems than synthetic lipid carriers. As a drug carrier, exosomes can maintain the activity of the drug within the membrane and can release it without triggering an immune response. Tumor therapy based on exosomes may become an important part of personalized medicine, as many studies on exosome as drug carriers used in therapy of tumors seem to show[154].

Exosomes can be loaded with different types of compounds, such as small-molecule chemical drugs, proteins and nucleic acids. Small-molecule chemical drugs usually have low solubility, high toxicity, and poor specificity. Therefore, exosomes were used for wrapping small-molecule chemical drugs to avoid these disadvantages and they can easily enter the tumors. For example, when paclitaxel with high concentration was added to the culture medium of MSCs derived from bone marrow, the MSCs took up the paclitaxel and released exosomes containing the drug, which were transported into pancreatic cancer cells to exert a targeted antitumor effect[155]. Exosomes derived from immature DC cells incubated with doxorubicin can be used for targeted therapy of breast cancer. Exosomes were first used to deliver adriamycin to solid tumors in mice and provided a new route for the delivery of hydrophobic chemotherapy drugs[156]. Exosomes were also used to deliver proteins for disease treatment. It was found that exosomes loaded with therapeutic cytosine deaminase-uracil phosphoribosyltransferase (CD-UPRT) mRNA/protein combined with 5-fluorouracil were able to significantly inhibit the growth of schwannomas in mice[157]. Exosomes loaded with siRNAs and miRNAs were used to target cells and induce genetic modification in biological and pathogenic processes. It is well known that siRNA has low stability and is easily degraded when unprotected in the circulation. Exosomes can be loaded with specific siRNAs to protect and deliver them to target cells. One study showed that exosomes derived from HeLa cells could deliver RAD51 siRNA into human cervical cancer cells and effectively induce their death, which proved the feasibility of using exosomes to transport siRNAs for antitumor treatment[158]. In hepatocellular carcinoma, exosomes derived from MSC transported the tumor suppressor miR-122 and induced autophagy and cell cycle arrest of HCC cells by changing the expression of its target genes (Table 2)[159].

Depending on how exosomes are used for drug transport, they can be divided into two categories: direct carriers and indirect carriers. In the direct carrier method, the drug is loaded into the exosomes by incubating them with the desired small-molecule compound[160]. Some drugs can also be directly loaded into exosomes by liposome transfection, which is mainly used for gene drugs. Drugs can also be transferred into the exosomes by electroporation, which makes a recoverable small hole in the phospholipid bilayer of exosome by applying a weak current pulse in an electrolyte solution. The direct carrier method is obviously convenient for drug loading. Both the size of the nucleic acid and the diameter of the exosomes played an important role in the loading of genetic drugs[161]. For protein drugs, saponin-assisted method can be used. Exosomes and drugs were incubated with saponin. Then, hypotonic dialysis was performed by transferring exosomes and drugs into dialysis membranes and purified by size-exclusion chromatography and finally obtained drug-loaded exosomes[162]. As indirect carriers, exosomes will be given their payload by manipulating the cells from which the exosomes are derived. The donor cells can be cultured in the presence of a drug with low cytotoxicity, which will be taken up and incorporated into the intracellular vesicles and subsequently appear in the secreted exosomes[163]. Nucleic acids for exosome loading can be transfected into the donor cells (Table 2 and Fig. 3)[164].

For use in cancer therapy, exosomes should be administrable by conventional routes and must maintain their stability in the body. Intravenous administration is the commonest method, but exosomes only exist in the blood circulation for a short time.

Table 2 Drug-loaded exosomes in cancer therapy.

Drug type	Drug loading method	Therapy
Small molecule chemical drugs	Drugs−exosomes co-cultivating	Paclitaxel-loaded exosomes target to pancreatic cancer
	Drugs−cells co-cultivating	Doxorubicin-loaded exosomes target to breast cancer
Proteins	Expression of target protein after transfection	CD-UPRT-loaded exosomes target to schwannomas
	Saponin-assisted	
Nucleic acids	Electroporation	RAD51 siRNA-loaded exosomes target to cervical cancer
	Liposome transfection	miR-122-loaded exosomes target to HCC

Studies in mice have shown that the half-life of intravenous exosomes was about 2 min; therefore, it is necessary to modify the exosomes to prevent them from being eliminated too quickly and increase the likelihood of their reaching the target cells[165]. One way to accomplish this is by injecting loaded exosomes directly into the tumor, but this only works for accessible cancers. Oral administration of exosomes can also be used. In a mouse lung cancer model, oral administration of paclitaxel-loaded exosomes suppressed the growth of lung cancer cells better than intraperitoneal paclitaxel[166]. Intraperitoneal, subcutaneous and intranasal administrations can also be used for delivery of exosomes[167,168].

To enhance the therapeutic effect of exosomes and reduce drug toxicity on healthy cells, it is necessary to improve the targeting of exosomes by modifying their surface proteins to recognize cognate molecules on the surface of the tumor cells. For example, immature DCs were modified by the fusion of αv integrin-specific iRGD peptide (CRGDKGPDC) with lysosome-associated membrane glycoprotein 2b (LAMP2b). The modified immature DCs secreted doxorubicin-loaded exosomes, which specifically recognized integrin $\alpha v\beta 3$ on breast cancer cells. These exosomes had a stronger antitumor effect than doxorubicin alone or doxorubicin in exosomes derived from unmodified DCs[156]. Alternatively, magnetic nanoparticles can be used to increase targeting of exosomes. Exosomes loaded with adriamycin and magnetic particles could be drawn to the tumor site in mice by the application of a magnetic field[169].

5. Additional studies on exosomes

Although the clinical application of exosomes is still in its infancy, some progress has been made in tumor diagnosis and therapy. In 2016, Exosome Diagnostics Company launched the world's first exosomal cancer diagnostic product ExoDx Lung (ALK), which utilized liquid biopsy technology to screen for specific exosomal RNAs in the blood. The method was sensitive and accurate, and could detect EML4-ALK mutations in patients with NSCLC. Since then, other diagnostic products such as ExoDx Lung (EGFR) have been successfully launched[170]. A clinical trial published in 2016 proved that HSP70 exosomes can be used for early diagnosis of malignant solid tumors, and the study was completed on April 8, 2019 (ClinicalTrials.gov Identifier: NCT02662621). A novel integrated microfluidic device has been specifically designed for isolation and *in situ* detection of lung cancer-specific exosomes collected from patient's urine. Urine samples from lung cancer patients and controls were used to validate this method, which showed great promise for differentiating early-stage lung cancer patients from healthy individuals[171]. Paul et al.[172] used high-resolution atomic force microscopy (AFM) and spectroscopy (AFS) techniques to demonstrate differences between EVs derived from colon cancer cells and colon epithelial cells at the single-vesicle level. Compared with normal cells, the EVs of colon cancer cells have a significantly increased surface density of hyaluronic acid (HA). This is the first report of HA-coated EVs used as a potential indicator of colon cancer.

For cancer treatment, Kamerkar et al.[41] explored the possibility of using exosomes to carry antitumor RNAi. Exosomes secreted by normal fibroblast-like mesenchymal cells were engineered to encapsulate siRNA or shRNA against the mutant KRAS G12D. The engineered exosomes (iExosomes) targeted KRAS G12D in mouse models of pancreatic cancer, inhibited tumor growth and significantly increased overall survival. Researchers submitted an investigational drug application to the FDA in 2018, and entered phase I clinical trials in March 2020 (ClinicalTrials.gov Identifier: NCT03608631). Gustave Roussy and the Curie Institutes have developed an immunotherapy technique involving metronomic cyclophosphamide (mCTX) followed by vaccinations with tumor antigen-loaded DEX. The mCTX inhibits Treg functions and the DEX activates innate and adaptive immunity (ClinicalTrials.gov Identifier: NCT01159288). Another study showed that sulfisoxazole, a FDA-approved oral antibiotic, inhibited exosome secretion from breast cancer cells through interference with endothelin receptor A, and showed significant antitumor and anti-metastatic effects against breast cancer xenografts in mouse models[173]. A prospective study evaluated the usefulness of PD-L1 screening in exosomes in melanoma patients during follow-up. Results showed that PD-L1 level in circulating exosomes was a more reliable marker than PD-L1 expression in tumor biopsies, and measurement of circulating exosomal PD-L1 may be useful for monitoring tumor response to treatment and predicting clinical outcomes[174]. Huang et al.[175] developed a homogeneous, low-volume, efficient, and sensitive exosomal PD-L1 (HOLMES-ExoPD-L1) quantitation method for cancer diagnosis and immunotherapy response prediction. The level of circulating exosomal PD-L1 detected by HOLMES-ExoPD-L1 can effectively distinguish cancer patients from healthy volunteers.

In the tumor organoid system, MMP3 affected tumor progression through EVs, and knockout of MMP3 weakened solid tumor organoids and secretion of cancer EVs[176]. Wang et al.[177] reported a novel 'cocktail therapy' strategy based on excess natural killer cell-derived exosomes (NKEXOs) in combination with biomimetic core−shell nanoparticles (NNs) for tumor-targeted therapy. The NN/NKEXO cocktail showed highly efficient targeting and therapeutic miRNA delivery to neuroblastoma cells *in vivo*, leading to dual inhibition of tumor growth. A recent study showed that disruption of CD47−SIRPa interaction by SIRPa-exosomes led to an increase in cells being engulfed by macrophages and a concomitant inhibition of tumor growth in tumor-bearing mice. SIRPa exosomes promoted an intensive T cell infiltration in syngeneic mouse models of cancer, raising the possibility of CD47-targeted therapies to unleash both an innate and adaptive antitumor response[178]. Exosomal miR-1910-3p promoted proliferation, metastasis, and autophagy of breast cancer cells by targeting MTMR3 and activating the NF-κB signaling

pathway. Serum miR-1910-3p in exosomes was an effective diagnostic marker for improving the sensitivity of breast cancer diagnosis when used in combination with the traditional tumor marker CA153[179]. A recent study showed that exosomes derived from bone marrow mesenchymal stem cells can act as PDAC-homing vehicles to surpass the restrictions of pathological ECM and increase the accumulation of therapeutics in tumor site. What's more, paclitaxel and gemcitabine monophosphate loaded exosomes helped to overcome chemoresistance of PDAC[180].

6. Conclusions and future prospects

In recent years, more and more studies have focused on exosomes and their roles in intercellular and intracellular communication. Understanding the physiological functions of exosomes is essential for clarifying how they influence cancer progression. Exosomes can play opposite effects in the immune response to tumors. They can help tumor cells to escape immune surveillance and develop immune tolerance whereas exosomes derived from immune cells can also inhibit growth, proliferation, and metastasis of tumor cells. Exosomes participate in many processes of tumor metastasis, including the epithelial−mesenchymal transition, tumor angiogenesis, remodeling of the extracellular matrix, organ-specific metastasis, and evading attack by the immune system. Exosomes can also promote drug resistance of cancers by many mechanisms.

In summary, exosomes can modulate the signal transduction pathways in cancers by involving in multiple processes related to initiation, development and metastasis. This capability provides us with a wealth of new approaches for cancer diagnosis and treatment. It is easy to obtain liquid biopsies and isolate exosomes from patients with cancer. In the future, more and more studies will be focused on exosomes in diagnosis of cancers at early stage. For treatment, safe and effective tumor vaccines can be prepared by utilizing the ability of exosomes to regulate the immune reaction in the tumor microenvironment and tumor antigen presentation. Furthermore, exosomes are natural drug carriers with targeting potential that can be exploited for killing cancer cells while leaving healthy cells untouched. Despite many advantages of exosomes, they still face some critical problems in clinical application, such as low targeting efficiency and easy phagocytosis by the immune system. The method of separation and purification of exosomes also has limitations, because it is time-consuming and laborious. Exosomes also exhibit much heterogeneity. The composition of exosomes differs depending on the cell type that produced them, and this must be controlled in the clinic. Therefore, more researches will have to be done to focus on solving these problems and developing more effective clinical applications of exosomes.

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Author contributions

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Conflicts of interest

The authors declare that there is no conflict of interests. All authors have reviewed the content in full and agreed on submission.

References

1. Trams EG, Lauter CJ, Salem Jr N, Heine U. Exfoliation of membrane *ecto*-enzymes in the form of micro-vesicles. *Biochim Biophys Acta* 1981;**645**:63−70.
2. Johnstone RM, Adam M, Hammond JR, Orr L, Turbide C. Vesicle formation during reticulocyte maturation. Association of plasma membrane activities with released vesicles (exosomes). *J Biol Chem* 1987;**262**:9412−20.
3. Liu Y, Wang Y, Lv Q, Li X. Exosomes: from garbage bins to translational medicine. *Int J Pharm* 2020;**583**:119333.
4. Kalluri R, LeBleu VS. The biology, function, and biomedical applications of exosomes. *Science* 2020;**367**:eaau6977.
5. Thery C, Zitvogel L, Amigorena S. Exosomes: composition, biogenesis and function. *Nat Rev Immunol* 2002;**2**:569−79.
6. Colombo M, Raposo G, Thery C. Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. *Annu Rev Cell Dev Biol* 2014;**30**:255−89.
7. Simons M, Raposo G. Exosome—vesicular carriers for intercellular communication. *Curr Opin Cell Biol* 2009;**21**:575−81.
8. Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee JJ, Lotvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat Cell Biol* 2007;**9**:654−9.
9. Gusachenko ON, Zenkova MA, Vlassov VV. Nucleic acids in exosomes: disease markers and intercellular communication molecules. *Biochemistry (Mosc)* 2013;**78**:1−7.
10. Sadik N, Cruz L, Gurtner A, Rodosthenous RS, Dusoswa SA, Ziegler O, et al. Extracellular RNAs: a new awareness of old perspectives. *Methods Mol Biol* 2018;**1740**:1−15.
11. Oliveira Jr GP, Zigon E, Rogers G, Davodian D, Lu S, Jovanovic-Talisman T, et al. Detection of extracellular vesicle RNA using molecular beacons. *iScience* 2020;**23**:100782.
12. Skotland T, Hessvik NP, Sandvig K, Llorente A. Exosomal lipid composition and the role of ether lipids and phosphoinositides in exosome biology. *J Lipid Res* 2019;**60**:9−18.
13. Wen SW, Lima LG, Lobb RJ, Norris EL, Hastie ML, Krumeich S, et al. Breast cancer-derived exosomes reflect the cell-of-origin phenotype. *Proteomics* 2019;**19**:e1800180.
14. Ferguson SW, Nguyen J. Exosomes as therapeutics: the implications of molecular composition and exosomal heterogeneity. *J Control Release* 2016;**228**:179−90.
15. Keerthikumar S, Chisanga D, Ariyaratne D, Al Saffar H, Anand S, Zhao K, et al. ExoCarta: a web-based compendium of exosomal cargo. *J Mol Biol* 2016;**428**:688−92.
16. Kim DK, Kang B, Kim OY, Choi DS, Lee J, Kim SR, et al. EVpedia: an integrated database of high-throughput data for systemic analyses of extracellular vesicles. *J Extracell Vesicles* 2013;**2**.
17. Pathan M, Fonseka P, Chitti SV, Kang T, Sanwlani R, Van Deun J, et al. Vesiclepedia 2019: a compendium of RNA, proteins, lipids and metabolites in extracellular vesicles. *Nucleic Acids Res* 2019;**47**:516−9.
18. Stoorvogel W, Strous GJ, Geuze HJ, Oorschot V, Schwartz AL. Late endosomes derive from early endosomes by maturation. *Cell* 1991;**65**:417−27.
19. Hanson PI, Cashikar A. Multivesicular body morphogenesis. *Annu Rev Cell Dev Biol* 2012;**28**:337−62.
20. Sahu R, Kaushik S, Clement CC, Cannizzo ES, Scharf B, Follenzi A, et al. Microautophagy of cytosolic proteins by late endosomes. *Dev Cell* 2011;**20**:131−9.

21. Colombo M, Moita C, van Niel G, Kowal J, Vigneron J, Benaroch P, et al. Analysis of ESCRT functions in exosome biogenesis, composition and secretion highlights the heterogeneity of extracellular vesicles. *J Cell Sci* 2013;**126**:5553−65.
22. Tamai K, Tanaka N, Nakano T, Kakazu E, Kondo Y, Inoue J, et al. Exosome secretion of dendritic cells is regulated by Hrs, an ESCRT-0 protein. *Biochem Biophys Res Commun* 2010;**399**:384−90.
23. Gross JC, Chaudhary V, Bartscherer K, Boutros M. Active Wnt proteins are secreted on exosomes. *Nat Cell Biol* 2012;**14**:1036−45.
24. Hoshino D, Kirkbride KC, Costello K, Clark ES, Sinha S, Grega-Larson N, et al. Exosome secretion is enhanced by invadopodia and drives invasive behavior. *Cell Rep* 2013;**5**:1159−68.
25. Baietti MF, Zhang Z, Mortier E, Melchior A, Degeest G, Geeraerts A, et al. Syndecan-syntenin-ALIX regulates the biogenesis of exosomes. *Nat Cell Biol* 2012;**14**:677−85.
26. Trajkovic K, Hsu C, Chiantia S, Rajendran L, Wenzel D, Wieland F, et al. Ceramide triggers budding of exosome vesicles into multivesicular endosomes. *Science* 2008;**319**:1244−7.
27. Hornick CA, Hamilton RL, Spaziani E, Enders GH, Havel RJ. Isolation and characterization of multivesicular bodies from rat hepatocytes: an organelle distinct from secretory vesicles of the Golgi apparatus. *J Cell Biol* 1985;**100**:1558−69.
28. Strauss K, Goebel C, Runz H, Mobius W, Weiss S, Feussner I, et al. Exosome secretion ameliorates lysosomal storage of cholesterol in Niemann-Pick type C disease. *J Biol Chem* 2010;**285**:26279−88.
29. Escola JM, Kleijmeer MJ, Stoorvogel W, Griffith JM, Yoshie O, Geuze HJ. Selective enrichment of tetraspan proteins on the internal vesicles of multivesicular endosomes and on exosomes secreted by human B-lymphocytes. *J Biol Chem* 1998;**273**:20121−7.
30. van Niel G, Charrin S, Simoes S, Romao M, Rochin L, Saftig P, et al. The tetraspanin CD63 regulates ESCRT-independent and -dependent endosomal sorting during melanogenesis. *Dev Cell* 2011;**21**:708−21.
31. Geminard C, de Gassart A, Blanc L, Vidal M. Degradation of AP2 during reticulocyte maturation enhances binding of Hsc70 and Alix to a common site on TFR for sorting into exosomes. *Traffic* 2004;**5**:181−93.
32. Kumar A, Deep G. Hypoxia in tumor microenvironment regulates exosome biogenesis: molecular mechanisms and translational opportunities. *Cancer Lett* 2020;**479**:23−30.
33. Stenmark H. Rab GTPases as coordinators of vesicle traffic. *Nat Rev Mol Cell Biol* 2009;**10**:513−25.
34. Savina A, Fader CM, Damiani MT, Colombo MI. Rab 11 promotes docking and fusion of multivesicular bodies in a calcium-dependent manner. *Traffic* 2005;**6**:131−43.
35. Hsu C, Morohashi Y, Yoshimura S, Manrique-Hoyos N, Jung S, Lauterbach MA, et al. Regulation of exosome secretion by Rab 35 and its GTPase-activating proteins TBC1D10A-C. *J Cell Biol* 2010;**189**:223−32.
36. Ostrowski M, Carmo NB, Krumeich S, Fanget I, Raposo G, Savina A, et al. Rab27a and Rab27b control different steps of the exosome secretion pathway. *Nat Cell Biol* 2010;**12**:19−30.
37. Zylbersztejn K, Galli T. Vesicular traffic in cell navigation. *FEBS J* 2011;**278**:4497−505.
38. Fader CM, Sanchez DG, Mestre MB, Colombo MI. TI-VAMP/-VAMP7 and VAMP3/cellubrevin: two v-SNARE proteins involved in specific steps of the autophagy/multivesicular body pathways. *Biochim Biophys Acta* 2009;**1793**:1901−16.
39. Proux-Gillardeaux V, Raposo G, Irinopoulou T, Galli T. Expression of the Longin domain of TI-VAMP impairs lysosomal secretion and epithelial cell migration. *Biol Cell* 2007;**99**:261−71.
40. Raposo G, Nijman HW, Stoorvogel W, Liejendekker R, Harding CV, Melief CJ, et al. B lymphocytes secrete antigen-presenting vesicles. *J Exp Med* 1996;**183**:1161−72.
41. Kamerkar S, LeBleu VS, Sugimoto H, Yang S, Ruivo CF, Melo SA, et al. Exosomes facilitate therapeutic targeting of oncogenic KRAS in pancreatic cancer. *Nature* 2017;**546**:498−503.
42. Parolini I, Federici C, Raggi C, Lugini L, Palleschi S, De Milito A, et al. Microenvironmental pH is a key factor for exosome traffic in tumor cells. *J Biol Chem* 2009;**284**:34211−22.
43. Zitvogel L, Regnault A, Lozier A, Wolfers J, Flament C, Tenza D, et al. Eradication of established murine tumors using a novel cell-free vaccine: dendritic cell-derived exosomes. *Nat Med* 1998;**4**:594−600.
44. Montecalvo A, Shufesky WJ, Stolz DB, Sullivan MG, Wang Z, Divito SJ, et al. Exosomes as a short-range mechanism to spread alloantigen between dendritic cells during T cell allorecognition. *J Immunol* 2008;**180**:3081−90.
45. Chen G, Huang AC, Zhang W, Zhang G, Wu M, Xu W, et al. Exosomal PD-L1 contributes to immunosuppression and is associated with anti-PD-1 response. *Nature* 2018;**560**:382−6.
46. Ning Y, Shen K, Wu Q, Sun X, Bai Y, Xie Y, et al. Tumor exosomes block dendritic cells maturation to decrease the T cell immune response. *Immunol Lett* 2018;**199**:36−43.
47. Crenshaw BJ, Gu L, Sims B, Matthews QL. Exosome biogenesis and biological function in response to viral infections. *Open Virol J* 2018;**12**:134−48.
48. Mashouri L, Yousefi H, Aref AR, Ahadi AM, Molaei F, Alahari SK. Exosomes: composition, biogenesis, and mechanisms in cancer metastasis and drug resistance. *Mol Cancer* 2019;**18**:75.
49. LeBleu VS, Kalluri R. Exosomes as a multicomponent biomarker platform in cancer. *Trends Cancer* 2020;**6**:767−74.
50. Kim H, Kim DW, Cho JAO. Exploring the key communicator role of exosomes in cancer microenvironment through proteomics. *Proteome Sci* 2019;**17**:5.
51. Greening DW, Gopal SK, Xu R, Simpson RJ, Chen W. Exosomes and their roles in immune regulation and cancer. *Semin Cell Dev Biol* 2015;**40**:72−81.
52. Whiteside TL. Exosomes and tumor-mediated immune suppression. *J Clin Invest* 2016;**126**:1216−23.
53. Gutierrez-Vazquez C, Villarroya-Beltri C, Mittelbrunn M, Sanchez-Madrid F. Transfer of extracellular vesicles during immune cell−cell interactions. *Immunol Rev* 2013;**251**:125−42.
54. Poggio M, Hu T, Pai CC, Chu B, Belair CD, Chang A, et al. Suppression of exosomal PD-L1 induces systemic anti-tumor immunity and memory. *Cell* 2019;**177**:414−27.
55. Kim JW, Wieckowski E, Taylor DD, Reichert TE, Watkins S, Whiteside TL. Fas ligand-positive membranous vesicles isolated from sera of patients with oral cancer induce apoptosis of activated T lymphocytes. *Clin Cancer Res* 2005;**11**:1010−20.
56. Chalmin F, Ladoire S, Mignot G, Vincent J, Bruchard M, Remy-Martin JP, et al. Membrane-associated Hsp 72 from tumor-derived exosomes mediates STAT3-dependent immunosuppressive function of mouse and human myeloid-derived suppressor cells. *J Clin Invest* 2010;**120**:457−71.
57. Ciravolo V, Huber V, Ghedini GC, Venturelli E, Bianchi F, Campiglio M, et al. Potential role of HER2-overexpressing exosomes in countering trastuzumab-based therapy. *J Cell Physiol* 2012;**227**:658−67.
58. Gao L, Wang L, Dai T, Jin K, Zhang Z, Wang S, et al. Tumor-derived exosomes antagonize innate antiviral immunity. *Nat Immunol* 2018;**19**:233−45.
59. Jella KK, Nasti TH, Li Z, Malla SR, Buchwald ZS, Khan MK. Exosomes, their biogenesis and role in inter-cellular communication, tumor microenvironment and cancer immunotherapy. *Vaccines (Basel)* 2018;**6**:69.
60. Shen M, Ren X. New insights into the biological impacts of immune cell-derived exosomes within the tumor environment. *Cancer Lett* 2018;**431**:115−22.
61. Viaud S, Terme M, Flament C, Taieb J, André F, Novault S, et al. Dendritic cell-derived exosomes promote natural killer cell activation and proliferation: a role for NKG2D ligands and IL-15Ralpha. *PLoS One* 2009;**4**:e4942.
62. Romagnoli GG, Zelante BB, Toniolo PA, Migliori IK, Barbuto JA. Dendritic cell-derived exosomes may be a tool for cancer immunotherapy by converting tumor cells into immunogenic targets. *Front Immunol* 2015;**5**:692.
63. Bell BM, Kirk ID, Hiltbrunner S, Gabrielsson S, Bultema JJ. Designer exosomes as next-generation cancer immunotherapy. *Nanomedicine* 2016;**12**:163−9.

64. He C, Zheng S, Luo Y, Wang B. Exosome theranostics: biology and translational medicine. *Theranostics* 2018;**8**:237−55.
65. Escudier B, Dorval T, Chaput N, André F, Caby MP, Novault S, et al. Vaccination of metastatic melanoma patients with autologous dendritic cell (DC) derived-exosomes: results of thefirst phase I clinical trial. *J Transl Med* 2005;**3**:10.
66. Morse MA, Garst J, Osada T, Khan S, Hobeika A, Clay TM, et al. A phase I study of dexosome immunotherapy in patients with advanced non-small cell lung cancer. *J Transl Med* 2005;**3**:9.
67. Tang XJ, Sun XY, Huang KM, Zhang L, Yang ZS, Zou DD, et al. Therapeutic potential of CAR-T cell-derived exosomes: a cell-free modality for targeted cancer therapy. *Oncotarget* 2015;**6**: 44179−90.
68. Hao S, Moyana T, Xiang J. Review: cancer immunotherapy by exosome-based vaccines. *Cancer Biother Radiopharm* 2007;**22**: 692−703.
69. Diepenbruck M, Christofori G. Epithelial−mesenchymal transition (EMT) and metastasis: yes, no, maybe?. *Curr Opin Cell Biol* 2016; **43**:7−13.
70. You Y, Shan Y, Chen J, Yue H, You B, Shi S, et al. Matrix metalloproteinase 13-containing exosomes promote nasopharyngeal carcinoma metastasis. *Cancer Sci* 2015;**106**:1669−77.
71. Franzen CA, Blackwell RH, Todorovic V, Greco KA, Foreman KE, Flanigan RC, et al. Urothelial cells undergo epithelial-to-mesenchymal transition after exposure to muscle invasive bladder cancer exosomes. *Oncogenesis* 2015;**4**:e163.
72. Vella LJ. The emerging role of exosomes in epithelial−mesenchymal transition in cancer. *Front Oncol* 2014;**4**:361.
73. Webber J, Steadman R, Mason MD, Tabi Z, Clayton A. Cancer exosomes trigger fibroblast to myofibroblast differentiation. *Cancer Res* 2010;**70**:9621−30.
74. Luga V, Wrana JL. Tumor-stroma interaction: revealing fibroblast-secreted exosomes as potent regulators of Wnt-planar cell polarity signaling in cancer metastasis. *Cancer Res* 2013;**73**:6843−7.
75. Cao M, Seike M, Soeno C, Mizutani H, Kitamura K, Minegishi Y, et al. MiR-23a regulates TGF-β-induced epithelial−mesenchymal transition by targeting E-cadherin in lung cancer cells. *Int J Oncol* 2012;**41**:869−75.
76. Zhang X, Sai B, Wang F, Wang L, Wang Y, Zheng L, et al. Hypoxic BMSC-derived exosomal miRNAs promote metastasis of lung cancer cells *via* STAT3-induced EMT. *Mol Cancer* 2019;**18**:40.
77. Wang L, Yang G, Zhao D, Wang J, Bai Y, Peng Q, et al. CD103-positive CSC exosome promotes EMT of clear cell renal cell carcinoma: role of remote MiR-19b-3p. *Mol Cancer* 2019;**18**:86.
78. Liao J, Liu R, Shi YJ, Yin LH, Pu YP. Exosome-shuttling microRNA-21 promotes cell migration and invasion-targeting PDCD4 in esophageal cancer. *Int J Oncol* 2016;**48**:2567−79.
79. Munagala R, Aqil F, Gupta RC. Exosomal miRNAs as biomarkers of recurrent lung cancer. *Tumour Biol* 2016;**37**:10703−14.
80. Pfeffer SR, Grossmann KF, Cassidy PB, Yang CH, Fan M, Kopelovich L, et al. Detection of exosomal miRNAs in the plasma of melanoma patients. *J Clin Med* 2015;**4**:2012−27.
81. Kucharzewska P, Christianson HC, Welch JE, Svensson KJ, Fredlund E, Ringnér M, et al. Exosomes reflect the hypoxic status of glioma cells and mediate hypoxia-dependent activation of vascular cells during tumor development. *Proc Natl Acad Sci U S A* 2013;**110**: 7312−7.
82. Horie K, Kawakami K, Fujita Y, Sugaya M, Kameyama K, Mizutani K, et al. Exosomes expressing carbonic anhydrase 9 promote angiogenesis. *Biochem Biophys Res Commun* 2017;**492**:356−61.
83. Wu F, Li F, Lin X, Xu F, Cui RR, Zhong JY, et al. Exosomes increased angiogenesis in papillary thyroid cancer microenvironment. *Biochem Biophys Res Commun* 2017;**492**:356−61.
84. Hood JL, San RS, Wickline SA. Exosomes released by melanoma cells prepare sentinel lymph nodes for tumor metastasis. *Cancer Res* 2011;**71**:3792−801.
85. Ludwig NAO, Yerneni SS, Razzo BM, Whiteside TAO. Exosomes from HNSCC promote angiogenesis through reprogramming of endothelial cells. *Mol Cancer Res* 2018;**16**:1798−808.
86. Gong M, Yu B, Wang J, Wang Y, Liu M, Paul C, et al. Mesenchymal stem cells release exosomes that transfer miRNAs to endothelial cells and promote angiogenesis. *Oncotarget* 2017;**8**:45200−12.
87. Pakravan K, Babashah S, Sadeghizadeh M, Mowla SJ, Mossahebi-Mohammadi M, Ataei F, et al. MicroRNA-100 shuttled by mesenchymal stem cell-derived exosomes suppresses *in vitro* angiogenesis through modulating the mTOR/HIF-1α/VEGF signaling axis in breast cancer cells. *Cell Oncol* 2017;**40**:457−70.
88. Wu Q, Zhou L, Lv D, Zhu X, Tang H. Exosome-mediated communication in the tumor microenvironment contributes to hepatocellular carcinoma development and progression. *J Hematol Oncol* 2019;**12**:53.
89. He M, Qin H, Poon TC, Sze SC, Ding X, Co NN, et al. Hepatocellular carcinoma-derived exosomes promote motility of immortalized hepatocyte through transfer of oncogenic proteins and RNAs. *Carcinogenesis* 2015;**36**:1008−18.
90. Hakulinen J, Sankkila L, Sugiyama N, Lehti K, Keski-Oja J. Secretion of active membrane type 1 matrix metalloproteinase (MMP-14) into extracellular space in microvesicular exosomes. *J Cell Biochem* 2008;**105**:1211−8.
91. Paget S. The distribution of secondary growths in cancer of the breast. 1889. *Cancer Metastasis Rev* 1989;**8**:98−101.
92. Hoshino A, Costa-Silva B, Shen TL, Rodrigues G, Hashimoto A, Tesic Mark M, et al. Tumour exosome integrins determine organotropic metastasis. *Nature* 2015;**527**:329−35.
93. Costa-Silva B, Aiello NM, Ocean AJ, Singh S, Zhang H, Thakur BK, et al. Pancreatic cancer exosomes initiate pre-metastatic niche formation in the liver. *Nat Cell Biol* 2015;**17**:816−26.
94. Steinbichler TB, Dudás J, Riechelmann H, Skvortsova II. The role of exosomes in cancer metastasis. *Semin Cancer Biol* 2017;**44**:170−81.
95. Liu Y, Gu Y, Han Y, Zhang Q, Jiang Z, Zhang X, et al. Tumor exosomal RNAs promote lung pre-metastatic niche formation by activating alveolar epithelial tlr3 to recruit neutrophils. *Cancer Cell* 2016;**30**:243−56.
96. Shedden K, Xie XT, Chandaroy P, Chang YT, Rosania GR. Expulsion of small molecules in vesicles shed by cancer cells: association with gene expression and chemosensitivity profiles. *Cancer Res* 2003;**63**:4331−7.
97. Bach DH, Hong JY, Park HJ, Lee SK. The role of exosomes and miRNAs in drug-resistance of cancer cells. *Int J Cancer* 2017;**141**: 220−30.
98. Yu DD, Wu Y, Shen HY, Lv MM, Chen WX, Zhang XH, et al. Exosomes in development, metastasis and drug resistance of breast cancer. *Cancer Sci* 2015;**106**:959−64.
99. Zhong S, Chen X, Wang D, Zhang X, Shen H, Yang S, et al. MicroRNA expression profiles of drug-resistance breast cancer cells and their exosomes. *Oncotarget* 2016;**7**:19601−9.
100. Wei Y, Lai X, Yu S, Chen S, Ma Y, Zhang Y, et al. Exosomal miR-221/222 enhances tamoxifen resistance in recipient ER-positive breast cancer cells. *Breast Cancer Res Treat* 2014;**147**:423−31.
101. Au Yeung CL, Co NN, Tsuruga T, Yeung TL, Kwan SY, Leung CS, et al. Exosomal transfer of stroma-derived miR 21 confers paclitaxel resistance in ovarian cancer cells through targeting APAF1. *Nat Commun* 2016;**7**:11150.
102. Santos JC, Lima NDS, Sarian LO, Matheu A, Ribeiro ML, Derchain SFM. Exosome-mediated breast cancer chemoresistance *via* miR-155 transfer. *Sci Rep* 2018;**8**:829.
103. Hu JL, Wang W, Lan XL, Zeng ZC, Liang YS, Yan YR, et al. CAFs secreted exosomes promote metastasis and chemotherapy resistance by enhancing cell stemness and epithelial−mesenchymal transition in colorectal cancer. *Mol Cancer* 2019;**18**:91.
104. Lei Y, Guo W, Chen B, Chen L, Gong J, Li W. Tumorreleased lncRNA H19 promotes gefitinib resistance *via* packaging into exosomes in nonsmall cell lung cancer. *Oncol Rep* 2018;**40**:3438−46.

105. Zhang Z, Yin J, Lu C, Wei Y, Zeng A, You Y. Exosomal transfer of long non-coding RNA SBF2-AS1 enhances chemoresistance to temozolomide in glioblastoma. *J Exp Clin Cancer Res* 2019;**38**:166.
106. Ning K, Wang T, Sun X, Zhang P, Chen Y, Jin J, et al. UCH-L1-containing exosomes mediate chemotherapeutic resistance transfer in breast cancer. *J Surg Oncol* 2017;**115**:932−40.
107. Corcoran C, Rani S, O'Brien K, O'Neill A, Prencipe M, Sheikh R, et al. Docetaxel-resistance in prostate cancer: evaluating associated phenotypic changes and potential for resistance transfer *via* exosomes. *PLoS One* 2012;**7**:e50999.
108. Koch R, Aung T, Vogel D, Chapuy B, Wenzel D, Becker S, et al. Nuclear trapping through inhibition of exosomal export by indomethacin increases cytostatic efficacy of doxorubicin and pixantrone. *Clin Cancer Res* 2016;**22**:395−404.
109. Liu J, Zhang Y, Liu A, Wang J, Li L, Chen X, et al. Distinct dasatinib-induced mechanisms of apoptotic response and exosome release in imatinib-resistant human chronic myeloid leukemia cells. *Int J Mol Sci* 2016;**17**:531.
110. Del Re M, Marconcini R, Pasquini G, Rofi E, Vivaldi C, Bloise F, et al. PD-L1 mRNA expression in plasma-derived exosomes is associated with response to anti-PD-1 antibodies in melanoma and NSCLC. *Br J Cancer* 2018;**118**:820−4.
111. Royo F, Zuñiga-Garcia P, Torrano V, Loizaga A, Sanchez-Mosquera P, Ugalde-Olano A, et al. Transcriptomic profiling of urine extracellular vesicles reveals alterations of CDH3 in prostate cancer. *Oncotarget* 2016;**7**:6835−46.
112. Gutkin A, Uziel O, Beery E, Nordenberg J, Pinchasi M, Goldvaser H, et al. Tumor cells derived exosomes contain hTERT mRNA and transform nonmalignant fibroblasts into telomerase positive cells. *Oncotarget* 2016;**7**:59173−88.
113. Yang JK, Song J, Huo HR, Zhao YL, Zhang GY, Zhao ZM, et al. DNM3, p65 and p53 from exosomes represent potential clinical diagnosis markers for glioblastoma multiforme. *Ther Adv Med Oncol* 2017;**9**:741−54.
114. Lawrie CH, Gal S, Dunlop HM, Pushkaran B, Liggins AP, Pulford K, et al. Detection of elevated levels of tumour-associated microRNAs in serum of patients with diffuse large B-cell lymphoma. *Br J Haematol* 2008;**141**:672−5.
115. van Niel G, D'Angelo G, Raposo G. Shedding light on the cell biology of extracellular vesicles. *Nat Rev Mol Cell Biol* 2018;**19**:213−28.
116. Taylor DD, Gercel-Taylor C. MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. *Gynecol Oncol* 2008;**110**:13−21.
117. Su YY, Sun L, Guo ZR, Li JC, Bai TT, Cai XX, et al. Upregulated expression of serum exosomal miR-375 and miR-1307 enhance the diagnostic power of CA125 for ovarian cancer. *J Ovarian Res* 2019;**12**:6.
118. Meng X, Müller V, Milde-Langosch K, Trillsch F, Pantel K, Schwarzenbach H. Diagnostic and prognostic relevance of circulating exosomal miR-373, miR-200a, miR-200b and miR-200c in patients with epithelial ovarian cancer. *Oncotarget* 2016;**7**:16923−35.
119. Jiao C, Jiao X, Zhu A, Ge J, Xu X. Exosomal miR-34s panel as potential novel diagnostic and prognostic biomarker in patients with hepatoblastoma. *J Pediatr Surg* 2017;**52**:618−24.
120. Sohn W, Kim J, Kang SH, Yang SR, Cho JY, Cho HC, et al. Serum exosomal microRNAs as novel biomarkers for hepatocellular carcinoma. *Exp Mol Med* 2015;**47**:e184.
121. He Y, Deng F, Yang S, Wang D, Chen X, Zhong S, et al. Exosomal microRNA: a novel biomarker for breast cancer. *Biomarkers Med* 2018;**12**:177−88.
122. Nedaeinia R, Manian M, Jazayeri MH, Ranjbar M, Salehi R, Sharifi M, et al. Circulating exosomes and exosomal microRNAs as biomarkers in gastrointestinal cancer. *Cancer Gene Ther* 2017;**24**:48−56.
123. Hosseini M, Khatamianfar S, Hassanian SM, Nedaeinia R, Shafiee M, Maftouh M, et al. Exosome-encapsulated microRNAs as potential circulating biomarkers in colon cancer. *Curr Pharmaceut Des* 2017;**23**:1705−9.
124. Tang Y, Zhao Y, Song X, Song X, Niu L, Xie L. Tumor-derived exosomal miRNA-320d as a biomarker for metastatic colorectal cancer. *J Clin Lab Anal* 2019;**33**:e23004.
125. Shi R, Wang PY, Li XY, Chen JX, Li Y, Zhang XZ, et al. Exosomal levels of miRNA-21 from cerebrospinal fluids associated with poor prognosis and tumor recurrence of glioma patients. *Oncotarget* 2015;**6**:26971−81.
126. Huang X, Yuan T, Liang M, Du M, Xia S, Dittmar R, et al. Exosomal miR-1290 and miR-375 as prognostic markers in castration-resistant prostate cancer. *Eur Urol* 2015;**67**:33−41.
127. Wani S, Kaul D, Mavuduru RS, Kakkar N, Bhatia A. Urinary-exosomal miR-2909: a novel pathognomonic trait of prostate cancer severity. *J Biotechnol* 2017;**259**:135−9.
128. Zhang ZY, Li YC, Geng CY, Zhou HX, Gao W, Chen WM. Serum exosomal microRNAs as novel biomarkers for multiple myeloma. *Hematol Oncol* 2019;**37**:409−17.
129. Hornick NI, Huan J, Doron B, Goloviznina NA, Lapidus J, Chang BH, et al. Serum exosome microRNA as a minimally-invasive early biomarker of AML. *Sci Rep* 2015;**5**:11295.
130. Zhao R, Zhang Y, Zhang X, Yang Y, Zheng X, Li X, et al. Exosomal long noncoding RNA HOTTIP as potential novel diagnostic and prognostic biomarker test for gastric cancer. *Mol Cancer* 2018;**17**:68.
131. Işın M, Uysaler E, Özgür E, Köseoğlu H, Şanlı Ö, Yücel Ö B, et al. Exosomal lncRNA-p21 levels may help to distinguish prostate cancer from benign disease. *Front Genet* 2015;**6**:168.
132. Zhan Y, Du L, Wang L, Jiang X, Zhang S, Li J, et al. Expression signatures of exosomal long non-coding RNAs in urine serve as novel non-invasive biomarkers for diagnosis and recurrence prediction of bladder cancer. *Mol Cancer* 2018;**17**:142.
133. Zheng R, Du M, Wang X, Xu W, Liang J, Wang W, et al. Exosome-transmitted long non-coding RNA PTENP1 suppresses bladder cancer progression. *Mol Cancer* 2018;**17**:143.
134. Lee YR, Kim G, Tak WY, Jang SY, Kweon YO, Park JG, et al. Circulating exosomal noncoding RNAs as prognostic biomarkers in human hepatocellular carcinoma. *Int J Cancer* 2019;**144**:1444−52.
135. Pan B, Qin J, Liu X, He B, Wang X, Pan Y, et al. Identification of serum exosomal HSA-circ-0004771 as a novel diagnostic biomarker of colorectal cancer. *Front Genet* 2019;**10**:1096.
136. Li Z, Yanfang W, Li J, Jiang P, Peng T, Chen K, et al. Tumor-released exosomal circular RNA PDE8A promotes invasive growth *via* the miR-338/MACC1/MET pathway in pancreatic cancer. *Cancer Lett* 2018;**432**:237−50.
137. Chen X, Chen RX, Wei WS, Li YH, Feng ZH, Tan L, et al. PRMT5 circular RNA promotes metastasis of urothelial carcinoma of the bladder through sponging miR-30c to induce epithelial-mesenchymal transition. *Clin Cancer Res* 2018;**24**:6319−30.
138. Elsharkasy OM, Nordin JZ, Hagey DW, de Jong OG, Schiffelers RM, Andaloussi SE, et al. Extracellular vesicles as drug delivery systems: why and how?. *Adv Drug Deliv Rev* 2020;**S0169−409X**:30024−7.
139. Wang L, Li Y, Guan X, Zhao J, Shen L, Liu J. Exosomal double-stranded DNA as a biomarker for the diagnosis and preoperative assessment of pheochromocytoma and paraganglioma. *Mol Cancer* 2018;**17**:128.
140. Sansone P, Savini C, Kurelac I, Chang Q, Amato LB, Strillacci A, et al. Packaging and transfer of mitochondrial DNA *via* exosomes regulate escape from dormancy in hormonal therapy-resistant breast cancer. *Proc Natl Acad Sci U S A* 2017;**114**:9066−75.
141. Jeppesen DK, Fenix AM, Franklin JL, Higginbotham JN, Zhang Q, Zimmerman LJ, et al. Reassessment of exosome composition. *Cell* 2019;**177**:428−45.
142. Melo SA, Luecke LB, Kahlert C, Fernandez AF, Gammon ST, Kaye J, et al. Glypican-1 identifies cancer exosomes and detects early pancreatic cancer. *Nature* 2015;**523**:177−82.
143. Sandfeld-Paulsen B, Aggerholm-Pedersen N, Bæk R, Jakobsen KR, Meldgaard P, Folkersen BH, et al. Exosomal proteins as prognostic biomarkers in non-small cell lung cancer. *Mol Oncol* 2016;**10**:1595−602.
144. Chen IH, Xue L, Hsu CC, Paez JS, Pan L, Andaluz H, et al. Phosphoproteins in extracellular vesicles as candidate markers for breast cancer. *Proc Natl Acad Sci U S A* 2017;**114**:3175−80.

145. Moon PG, Lee JE, Cho YE, Lee SJ, Jung JH, Chae YS, et al. Identification of developmental endothelial locus-1 on circulating extracellular vesicles as a novel biomarker for early breast cancer detection. *Clin Cancer Res* 2016;**22**:1757−66.
146. Huang K, Fang C, Yi K, Liu X, Qi H, Tan Y, et al. The role of PTRF/Cavin1 as a biomarker in both glioma and serum exosomes. *Theranostics* 2018;**8**:1540−57.
147. Sun Y, Liu S, Qiao Z, Shang Z, Xia Z, Niu X, et al. Systematic comparison of exosomal proteomes from human saliva and serum for the detection of lung cancer. *Anal Chim Acta* 2017;**982**:84−95.
148. Elsherbini A, Bieberich E. Ceramide and exosomes: a novel target in cancer biology and therapy. *Adv Cancer Res* 2018;**140**:121−54.
149. Skotland T, Ekroos K, Kauhanen D, Simolin H, Seierstad T, Berge V, et al. Molecular lipid species in urinary exosomes as potential prostate cancer biomarkers. *Eur J Cancer* 2017;**70**:122−32.
150. Puhka M, Takatalo M, Nordberg ME, Valkonen S, Nandania J, Aatonen M, et al. Metabolomic profiling of extracellular vesicles and alternative normalization methods reveal enriched metabolites and strategies to study prostate cancer-related changes. *Theranostics* 2017;**7**:3824−41.
151. Tao L, Zhou J, Yuan C, Zhang L, Li D, Si D, et al. Metabolomics identifies serum and exosomes metabolite markers of pancreatic cancer. *Metabolomics* 2019;**15**:86.
152. Cho S, Yang HC, Rhee WJ. Simultaneous multiplexed detection of exosomal microRNAs and surface proteins for prostate cancer diagnosis. *Biosens Bioelectron* 2019;**146**:111749.
153. Guo Q, Jiang C. Delivery strategies for macromolecular drugs in cancer therapy. *Acta Pharm Sin B* 2020;**10**:979−86.
154. Srivastava A, Babu A, Filant J, Moxley KM, Ruskin R, Dhanasekaran D, et al. Exploitation of exosomes as nanocarriers for gene-, chemo-, and immune-therapy of cancer. *J Biomed Nanotechnol* 2016;**12**:1159−73.
155. Pascucci L, Coccè V, Bonomi A, Ami D, Ceccarelli P, Ciusani E, et al. Paclitaxel is incorporated by mesenchymal stromal cells and released in exosomes that inhibit *in vitro* tumor growth: a new approach for drug delivery. *J Control Release* 2014;**192**:262−70.
156. Tian Y, Li S, Song J, Ji T, Zhu M, Anderson GJ, et al. A doxorubicin delivery platform using engineered natural membrane vesicle exosomes for targeted tumor therapy. *Biomaterials* 2014;**35**:2383−90.
157. Mizrak A, Bolukbasi MF, Ozdener GB, Brenner GJ, Madlener S, Erkan EP, et al. Genetically engineered microvesicles carrying suicide mRNA/protein inhibit schwannoma tumor growth. *Mol Ther* 2013;**21**:101−8.
158. Shtam TA, Kovalev RA, Varfolomeeva EY, Makarov EM, Kil YV, Filatov MV. Exosomes are natural carriers of exogenous siRNA to human cells *in vitro*. *Cell Commun Signal* 2013;**11**:88.
159. Lou G, Song X, Yang F, Wu S, Wang J, Chen Z, et al. Exosomes derived from miR-122-modified adipose tissue-derived MSCs increase chemosensitivity of hepatocellular carcinoma. *J Hematol Oncol* 2015;**8**:122.
160. Zhang HG, Kim H, Liu C, Yu S, Wang J, Grizzle WE, et al. Curcumin reverses breast tumor exosomes mediated immune suppression of NK cell tumor cytotoxicity. *Biochim Biophys Acta* 2007;**1773**:1116−23.
161. Lamichhane TN, Raiker RS, Jay SM. Exogenous DNA loading into extracellular vesicles *via* electroporation is size-dependent and enables limited gene delivery. *Mol Pharm* 2015;**12**:3650−7.
162. Fuhrmann G, Serio A, Mazo M, Nair R, Stevens MM. Active loading into extracellular vesicles significantly improves the cellular uptake and photodynamic effect of porphyrins. *J Control Release* 2015;**205**:35−44.
163. Zhuang X, Xiang X, Grizzle W, Sun D, Zhang S, Axtell RC, et al. Treatment of brain inflammatory diseases by delivering exosome encapsulated anti-inflammatory drugs from the nasal region to the brain. *Mol Ther* 2011;**19**:1769−79.
164. Alvarez-Erviti L, Seow Y, Yin H, Betts C, Lakhal S, Wood MJA. Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. *Nat Biotechnol* 2011;**29**:341−5.
165. Takahashi Y, Nishikawa M, Shinotsuka H, Matsui Y, Ohara S, Imai T, et al. Visualization and *in vivo* tracking of the exosomes of murine melanoma B16-BL6 cells in mice after intravenous injection. *J Biotechnol* 2013;**165**:77−84.
166. Agrawal AK, Aqil F, Jeyabalan J, Spencer WA, Beck J, Gachuki BW, et al. Milk-derived exosomes for oral delivery of paclitaxel. *Nanomedicine* 2017;**13**:1627−36.
167. Long Q, Upadhya D, Hattiangady B, Kim DK, An SY, Shuai B, et al. Intranasal MSC-derived A1-exosomes ease inflammation, and prevent abnormal neurogenesis and memory dysfunction after status epilepticus. *Proc Natl Acad Sci U S A* 2017;**114**:3536−45.
168. Wiklander OP, Nordin JZ, O'Loughlin A, Gustafsson Y, Corso G, Mäger I, et al. Extracellular vesicle *in vivo* biodistribution is determined by cell source, route of administration and targeting. *J Extracell Vesicles* 2015;**4**:26316.
169. Qi H, Liu C, Long L, Ren Y, Zhang S, Chang X, et al. Blood exosomes endowed with magnetic and targeting properties for cancer therapy. *ACS Nano* 2016;**10**:3323−33.
170. Castellanos-Rizaldos E, Zhang X, Tadigotla VR, Grimm DG, Karlovich C, Raez LE, et al. Exosome-based detection of activating and resistance EGFR mutations from plasma of non-small cell lung cancer patients. *Oncotarget* 2019;**10**:2911−20.
171. Yang Q, Cheng L, Hu L, Lou D, Zhang T, Li J, et al. An integrative microfluidic device for isolation and ultrasensitive detection of lung cancer-specific exosomes from patient urine. *Biosens Bioelectron* 2020;**163**:112290.
172. Paul D, Roy A, Nandy A, Datta B, Borar P, Pal SK, et al. Identification of biomarker hyaluronan on colon cancer extracellular vesicles using correlative afm and spectroscopy. *J Phys Chem Lett* 2020;**11**:5569−76.
173. Im EJ, Lee CH, Moon PG, Rangaswamy GG, Lee B, Lee JM, et al. Sulfisoxazole inhibits the secretion of small extracellular vesicles by targeting the endothelin receptor A. *Nat Commun* 2019;**10**:1387.
174. Cordonnier M, Nardin C, Chanteloup G, Derangere V, Algros MP, Arnould L, et al. Tracking the evolution of circulating exosomal-PD-L1 to monitor melanoma patients. *J Extracell Vesicles* 2020;**9**:1710899.
175. Huang M, Yang J, Wang T, Song J, Xia J, Wu L, et al. Homogeneous, low-volume, efficient, and sensitive quantitation of circulating exosomal PD-L1 for cancer diagnosis and immunotherapy response prediction. *Angew Chem Int Ed Engl* 2020;**59**:4800−5.
176. Taha EA, Sogawa C, Okusha Y, Kawai H, Oo MW, Elseoudi A, et al. Knockout of MMP3 weakens solid tumor organoids and cancer extracellular vesicles. *Cancers* 2020;**12**:1260.
177. Wang G, Hu W, Chen H, Shou X, Ye T, Xu Y. Cocktail strategy based on NK cell-derived exosomes and their biomimetic nanoparticles for dual tumor therapy. *Cancers* 2019;**11**:1560.
178. Koh E, Lee EJ, Nam GH, Hong Y, Cho E, Yang Y, et al. Exosome-SIRPα, a CD47 blockade increases cancer cell phagocytosis. *Biomaterials* 2017;**121**:121−9.
179. Wang B, Mao JH, Wang BY, Wang LX, Wen HY, Xu LJ, et al. Exosomal miR-1910-3p promotes proliferation, metastasis, and autophagy of breast cancer cells by targeting MTMR3 and activating the NF-κB signaling pathway. *Cancer Lett* 2020;**489**:87−99.
180. Zhou Y, Zhou W, Chen X, Wang Q, Li C, Chen Q, et al. Bone marrow mesenchymal stem cells-derived exosomes for penetrating and targeted chemotherapy of pancreatic cancer. *Acta Pharm Sin B* 2020;**10**:1563−75.