

Clinical significance of exosomal miRNAs and proteins in three human cancers with high mortality in China (Review)

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Abstract. Cancer is the second leading cause of mortality worldwide. More importantly, the mortality rates for cancer are increasing. In China, lung cancer, liver cancer and gastric cancer are the top three leading causes of mortality in males, whereas lung cancer, gastric cancer and liver cancer are ranked the top three causes of mortality in females. Exosomes are extracellular vesicles that are produced and released by many different cells; these vesicles have a size range between 30 and 100 nm in diameter, and contain a lipid bilayer. Exosomes exist in various bodily fluids, contain plentiful amounts of nucleic acids and proteins, and shuttle these materials between cells to mediate the development of cancers. The present review summarizes the composition of exosomes and methods for their isolation and then intensively highlights the latest findings on the contributions of exosomal microRNAs (miRNAs) and proteins to lung cancer, liver cancer and gastric cancer. Taken together, exosomal miRNAs and proteins may be used as noninvasive, novel biomarkers for cancer diagnosis, prognosis or precision treatment owing to their ability to promote tumor progression and metastasis, and their ability to regulate the immune response and tumor cell sensitivity to chemotherapy drugs.

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1. Introduction

Cancer is the second leading cause of death globally (1). In China, the numbers of newly diagnosed cases and deaths were approximately 3.0 million and 1.9 million, respectively, in 2010 (2). According to 2013 data, lung cancer, liver cancer and gastric cancer are the top three leading causes of mortality in males in China, whereas lung cancer, gastric cancer and liver cancer are the top three leading causes of mortality in females (3) (Fig. 1).

A growing number of studies have focused on the biology, function and clinical implications of exosomes in cancers (4,5), and it has been demonstrated that exosomal miRNAs and proteins can act as tumor biomarkers for clinical diagnosis or prognosis and that exosomes shuttle between cells to exchange genetic material, which promotes tumor progression, metastasis and prognosis; regulates the immune response; and affects the sensitivity of tumor cells to chemotherapy drugs (6-8). Therefore, exosomal miRNAs and proteins potentially play critical roles in cancers with high mortality rates.

2. Exosome composition

Exosomes are extracellular vesicles (EVs) that are produced and released by many different cells; and these vesicles range in size from 30 to 100 nm in diameter and contain a lipid bilayer (9,10). Proteins, DNA, mRNAs, miRNAs and lipids are enriched in exosomes (11). Exosomes transfer nucleic acids and proteins between different cells, leading to both the transportation of materials and cell-cell communication (6,12,13).

A set of distinct proteins are contained in exosomes (14), including heat-shock proteins (Hsp70, Hsp90), tetraspanins (CD9, CD81), ESCRT-related proteins (Alix, Tsg101), cytoskeletal proteins (actin, Tubulin) and GTPases (EEF1A1,

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EEF2) (15,16). These proteins are known to be involved in biogenesis, the sorting and secretion of exosomes (17), antigen presentation, the organization of membrane microdomains, the cytoskeleton, and the endosomal system (18,19). Typically, exosomes contain both cell-type specific proteins and proteins that are expressed in various cell types (20).

In addition to proteins, exosomes contain a significant amount of nucleic acids, including DNA, mRNAs, miRNAs, circular RNAs (circRNAs) and long noncoding RNAs (lncRNAs) (21). Of these, miRNAs are a class of well-known regulatory molecules that control posttranscriptional gene regulation (22). Increasing evidence has shown that exosomal miRNAs are associated with many diseases, such as cancers, diabetes and obesity (23-26). Interestingly, the miRNA content of exosomes is similar to that of the original tumor; thus, a series of studies has focused on exosomal miRNA profiles for cancer diagnostics (16). In particular, the shuttling of miRNAs may act as a tumor promoter or a tumor suppressor during tumorigenesis (27). Previous studies have uncovered exosomal miRNAs that are closely associated with tumorigenesis, metastasis and drug resistance in various kinds of cancers (28,29). All of these findings suggest that exosomal miRNAs play a pivotal role in the diagnosis, treatment and prognosis of cancers (30,31).

Additionally, cholesterol, diglycerides, phospholipids, glycerophospholipids, sphingomyelins and ceramides are enriched in exosomes (32). These lipids participate in exosome biogenesis, function and release. For example, the cellular trafficking of the tetraspanin CD82 to endosomes is regulated by the cholesterol content of the membrane, and ceramides can protect miRNAs from degradation by circulating RNases and govern the cellular distribution of the tetraspanin CD81. In addition, bioactive lipids such as prostaglandins, leukotrienes, fatty acids and lipid-related enzymes such as phospholipases A2 have been detected in exosomes (33).

3. Exosome isolation

Exosomes secreted by various types of living cells have been detected in a diverse range of bodily fluids, including peripheral blood, saliva, cerebrospinal fluid, ascites fluid, amniotic fluid, urine, breast milk and semen (31,34) (Fig. 2). It is clear that the utility of exosomes goes beyond basic research and extends to clinical practice. For this reason, an efficient and accurate method for exosome isolation is crucial.

Here, we compare the common methods for exosome isolation (Table I), including ultracentrifugation (UC), ultrafiltration (UF), immunomagnetic beads, size exclusion chromatography (SEC) and ExoQuick™ (35,36). UC is a common and simple method (37); however, recent studies indicated that more contaminants were found in exosomes isolated by UC compared to other methods mainly due to the presence of albumins. Furthermore, the high-velocity ultracentrifugation process could cause some exosomes to rupture, resulting in exosome loss (38). Recently, the challenges of UC approach have been again discussed, the conventional biophysical UC cannot distinguish exosomes from lipoproteins and oncosomes, other types of small EVs with sedimentation velocities and gradient densities similar to those of exosomes (39). UF does not require special equipment, although it leads to a reduction in the

membranes' lifespan and a low isolation efficiency (35,40). The use of immunomagnetic beads is an alternative method with high specificity and purity, but it is limited to exosomes with a known antigen and has a high reagent cost (35). Although SEC does not lead to significant albumin contamination, the efficiency is low (35,37,41). ExoQuick™ produces excellent reproducibility and sensitivity. However, the proprietary reagents exhibit contamination from unknown sources, and the polymer leads to protein aggregation (35,36,42,43). Moreover, the ExoQuick™ kit does not specifically precipitate exosomes, which means that other types of nanovesicles with similar sizes (30-100 nm) might also be coprecipitated (39). Recently, a new technique developed by the microfluidics community has been used to approach some of the problems with exosome isolation mentioned above. The most important feature of this method is exosome enrichment during isolation, which is beneficial for the detection of early-stage cancers. This microfluidics approach showed a superior recovery of 60-80% compared to the conventional techniques of UC (6%) and ExoQuick™ (30%) based on nanoparticle tracking analysis (NTA) (43).

Indeed, the high quantity and purity of exosomes are extremely important for exosomal biology studies. Thus, western blotting should be used to determine whether exosomal protein markers (Alix, Tsg101, Hsp70 or others) are present in exosome isolations (44). Simultaneously, transmission electron microscopy (TEM) is often utilized to observe exosome morphology, NTA is used to measure particle size, and the bicinchoninic acid assay (BCA) is performed to examine the protein concentration of exosomes (45). Additionally, to ensure the sensitivity of isolations and achieve a robust result, pre-analytical factors should be taken into consideration (Table II) (46,47).

4. Exosomal miRNAs and proteins in lung cancer

The latest report showed that lung cancer caused approximately 597,000 deaths in China in 2013 (3). Of lung cancer cases, approximately 95% are non-small-cell lung cancer (NSCLC) and small-cell lung cancer (SCLC) (48), which together represent the most common cause of cancer-related death globally (49,50).

Serving as biomarkers. Exosomes and exosomal miRNAs differed between patients with lung cancer and controls (51). By comparing 12 specific tumor- and exosome-derived miRNAs (miR-17-3p, miR-21, miR-106a, miR-146, miR-155, miR-191, miR-192, miR-203, miR-205, miR-210, miR-212, and miR-214) in lung cancer, previous studies revealed that there was no significant difference between circulating miRNAs and tumor miRNAs, demonstrating that exosome-derived miRNAs can serve as diagnostic biomarkers for lung cancer (51). In a nude mouse model of subcutaneous primary and recurrent lung cancer xenografts *in vivo*, miR-21 and miR-155 were found to be up-regulated in serum exosomes derived from recurrent tumor-bearing nude mice compared to nontumor- or primary tumor-bearing nude mice (52), suggesting that these two miRNAs might be potential prognostic biomarkers for noninvasive diagnosis of recurrent lung cancer. In addition, Liu *et al* (53) first reported that elevation of plasma exosomal miR-23b-3p, miR-10b-5p and miR-21-5p predicted a significantly poor

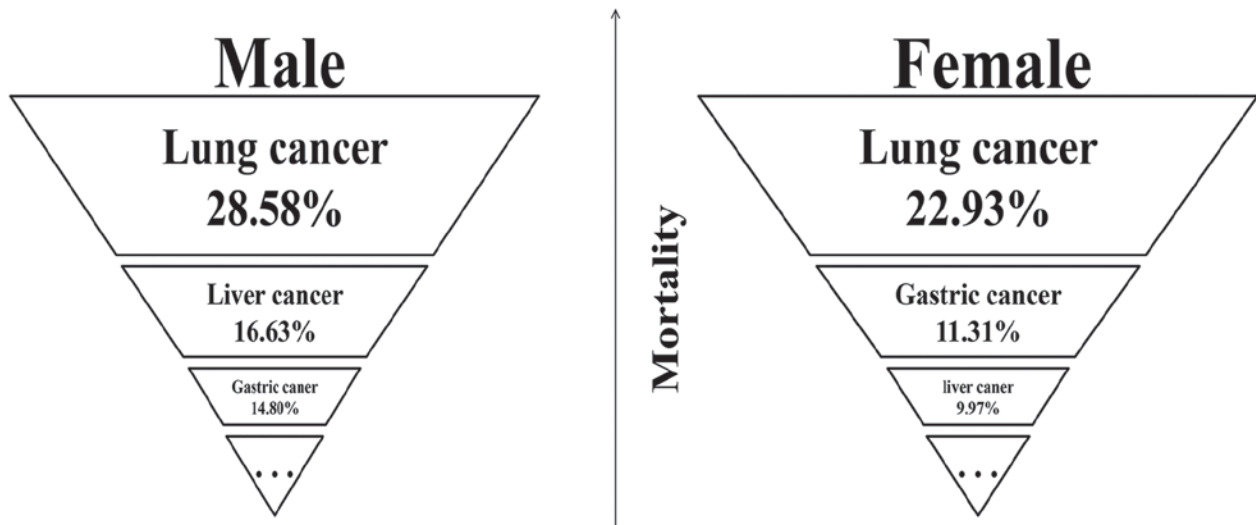


Figure 1. Top three mortality cancers in China, 2013. Liver cancer and gastric cancer are the top three leading causes of mortality in males. Lung cancer, gastric cancer and liver cancer are ranked the top three causes of mortality in females.

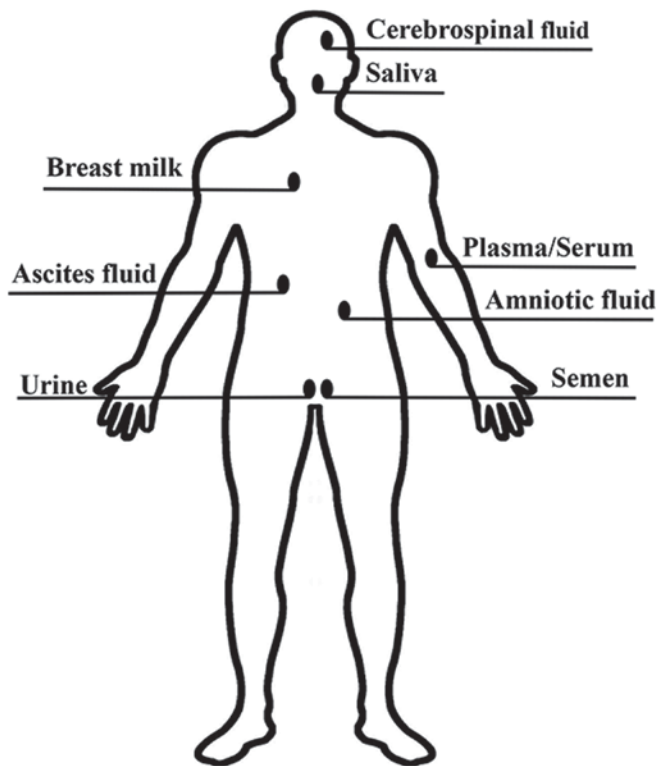


Figure 2. Exosomes exist in various bodily fluids. Exosomes can be detected in peripheral blood, saliva, cerebrospinal fluid, ascites fluid, amniotic fluid, urine, breast milk, semen and other bodily fluids.

survival, implying that these three exosomal miRNAs could serve as independent prognostic biomarkers for NSCLC.

Exosomal membrane-bound proteins, for example, the epidermal growth factor receptor (EGFR), NY-ESO-1 and CD91, are also promising diagnostic or prognostic biomarker candidates for lung cancer. Yamashita *et al* (54) demonstrated that the measurement of plasma exosomal proteins might be helpful for *in vitro* diagnosis, and exosomal EGFR was a potential diagnostic biomarker for the characterization of lung cancer. In NSCLC patients, exosomal NY-ESO-1 was a strong

prognostic biomarker of poorer survival (55). CD91 expression was significantly increased in serum exosomes derived from patients with lung adenocarcinoma (ADC), and its detection power for early-stage patients was higher than that of carcino-embryonic antigen (CEA) (56).

Stimulating angiogenesis and inducing metastasis. Angiogenesis is essential for tumor growth, progression and metastasis (57). Liu *et al* (58) found that exosomal miR-21 derived from cigarette smoke extract (CSE)-transformed human bronchial epithelial (HBE) cells was elevated, and this increased exosomal miR-21 led to STAT3 activation and altered the vascular endothelial growth factor (VEGF) expression of recipient cells, promoting CSE-induced angiogenesis and the malignant transformation of HBE cells. These results provided a novel intervention strategy to prevent carcinogenesis of lung cancer. In addition, hypoxic lung cancer cell (hypoxic CL1-5)-derived exosomal miR-23a enhanced neovascularization and tumor growth, and serum exosomal miR-23a was also elevated in patients with lung cancer. These findings provided strong evidence that an increase in exosomal miR-23a contributes to angiogenesis, intravasation and extravasation in lung cancer (59).

Exosomes play a fundamental role in the premetastatic niche and metastasis (4). Results from Fabbri *et al* (60) indicated that miRNAs (miR-21/29a) derived from lung cancer cell line (A549 and SK-MES) exosomes activate members of the Toll-like receptor (TLR) family (murine TLR7 and human TLR8) in immune cells, leading to a TLR-mediated prometastatic inflammatory response that might ultimately trigger tumor growth and metastasis.

Mediating cisplatin (DDP) resistance. Lung cancer cell-derived exosomes could confer DDP resistance to other cancer cells. Qin *et al* (61) established A549 cells that were resistant to DDP (A549/DDP). Compared with A549 exosomes, miR-100-5p was downregulated by 75% in A549/DDP cell exosomes. Lower expression of miR-100-5p induced DDP resistance in recipient cells (other lung cancer cell lines). miR-100-5p negatively regulated mTOR, the mammalian target of

Table I. Comparison of exosome isolation methods.

Author, year	Method	Principle	Advantages	Disadvantages	(Refs.)
Baranyai <i>et al</i> , 2015; Peterson <i>et al</i> , 2015	UC	Separating the exosomes through differential mass, density and shape	<ul style="list-style-type: none"> • Available technology • Simple operation 	<ul style="list-style-type: none"> • The high velocity ultracentrifugation process could cause some exosomes rupture that results in some exosomes loss • Contaminated with albumin and IgG • Time consuming (16-20 h) 	(37,38)
Li <i>et al</i> , 2017; Zeringer <i>et al</i> , 2015	UF	Depending on exosomal size or molecular weight	<ul style="list-style-type: none"> • No need of special equipment • Good portability 	<ul style="list-style-type: none"> • Clogging and vesicle trapping lead to reduce the membranes' lifetime and low isolation efficiency 	(35,40)
Li <i>et al</i> , 2017	Immunomagnetic beads	Specific exosomal antigens (receptors) can be captured by magnetic beads (ligands)	<ul style="list-style-type: none"> • High specificity and purity • No damage on the integrity of the exosomes' morphology and structure 	<ul style="list-style-type: none"> • High reagent cost • Low yield 	(35)
Li <i>et al</i> , 2017; Baranyai <i>et al</i> , 2015; Taylor and Shah, 2015	SEC	A porous stationary phase is utilized to sort exosomes out according to the size	<ul style="list-style-type: none"> • Obtaining high-purity exosomes without significant albumin contamination • Excellent reproducibility and sensitivity 	<ul style="list-style-type: none"> • Require dedicated equipment • Low efficiency 	(35,37,41)
Li <i>et al</i> , 2017; Caradec <i>et al</i> , 2014; Ban <i>et al</i> , 2015	ExoQuick™	By the precipitation approach	<ul style="list-style-type: none"> • Efficient (around 100%) and reproducible • Decreasing albumin contamination • Fast (within 30 min) 	<ul style="list-style-type: none"> • Isolation procedure should be under acidic conditions (pH=4) • Polymer precipitates protein aggregation 	(35,36,42)

UC, ultracentrifugation; UF, ultrafiltration; SEC, size exclusion chromatography.

rapamycin, to alter the recipient lung cancer cells' resistance to DDP. Additionally, the chemosensitivity of NSCLC to DDP could be regulated by serum exosomal miR-146a-5p. The overexpression of miR-146a-5p reversed the resistance of A549/DDP cells by targeting Atg12 to inhibit autophagy (62). Furthermore, in a human bronchial epithelial cell (HBEC) model, exosomes derived from chemoresistant mesenchymal NSCLC cells were able to transfer chemoresistance and mesenchymal phenotypes to recipient cells, thereby enhancing resistance to gemcitabine and cisplatin/gemcitabine combination therapy (63).

5. Exosomal miRNAs and proteins in liver cancer

Liver cancer is a common malignancy with a high mortality rate both in China and around the world (64,65). Liver cancer includes primary liver cancer (PLC) and secondary liver

cancer. Hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (ICC) are two different histologic types of PLC, which is the second most common cause of cancer-related deaths worldwide (66).

Serving as biomarkers. Differential expression of exosomal miRNAs in serum could serve as a diagnostic biomarker for HCC. Sohn *et al* (67) reported that the levels of serum exosomal miR-18a, miR-221, miR-222 and miR-224 were remarkably higher in HCC patients compared with patients with liver cirrhosis (LC) or chronic hepatitis B (CHB); however, the levels of serum exosomal miR-101, miR-106b, miR-122 and miR-195 were lower in HCC patients than in CHB patients. In addition, other studies have shown that expression of exosomal miR-21 and miR-125b was upregulated in HCC patients compared with CHB patients or healthy controls. More importantly, the levels of miR-21

Table II. Pre-analytical considerations.

Author, year	Pre-analytical considerations	(Refs.)
Muller <i>et al</i> , 2014; Witwer <i>et al</i> , 2013	Venous blood from patients or healthy volunteers is collected into tubes without heparin-based anticoagulants, EDTA may be more appropriate.	(46,47)
Witwer <i>et al</i> , 2013	Blood should be processed quickly at room temperature.	(47)
Witwer <i>et al</i> , 2013	Collected blood should be handled gently and tubes should be vertically positioned prior to centrifugation.	(47)
Witwer <i>et al</i> , 2013	Both plasma and serum can be used, but most studies indicate the isolation of exosomes prefers to plasma.	(47)
Muller <i>et al</i> , 2014	Harvested plasma or serum should be immediately used or stored at -80°C.	(46)

and miR-125b were higher in exosomes than in serum samples (68,69).

Promoting proliferation, invasion and metastasis. Exosomal miRNAs could affect cellular gene expression and cellular behaviors in target cells (70). Wei *et al* (71) showed that exosomes derived from HCC cells (SMMC-7721, Hep3B, and Huh-7) could functionally deliver miRNAs to target cells and that Vps4A regulated the secretion and uptake of these miRNAs in hepatoma cells by utilizing exosomes as mediators. Vps4A-associated miRNAs are believed to regulate the PI3K/AKT signaling pathway and promote the proliferation, invasion and metastasis of HCC cells. It has been suggested that a large number of protumorigenic RNAs and proteins, such as the MET proto-oncogene, caveolins (CAV1, CAV2) and an S100 family member (S100A4), are enriched in metastatic HCC-derived exosomes (72-74). Moreover, He *et al* (75) showed that uptake of these shuttling molecules in exosomes derived from motile HCC cell lines (HKCI-C3, HKCI-8 and MHCC97 L) markedly enhanced the invasive and migratory abilities of nonmotile immortalized hepatocyte (MIHA) cell lines by activating the PI3K/AKT and MAPK signaling pathways and increasing the secretion of matrix metalloproteinases (MMP)-2 and MMP-9, which induced cell invasion.

Mediating sensitivity to sorafenib. Sorafenib is predominantly used for the treatment of liver cancer and can improve the overall survival of patients with advanced HCC (76). Exosomes may mediate sorafenib resistance in HCC cells. Guo *et al* (77) revealed that miR-122 contained in adipose tissue mesenchymal stem cell (AMSC) exosomes enhanced HCC cell sensitivity to chemotherapeutic agents. Compared with the control groups, the inhibitory effect of 5-fluorouracil (5-FU) or sorafenib on HCC cells (HepG2 and Huh7) treated with AMSC-derived exosomes (122-Exo) was significantly enhanced, thereby providing a new strategy for HCC therapy. An important mechanism of sorafenib resistance is the over-expression of c-Met, a proto-oncogene that serves as a receptor for hepatocyte growth factor (HGF) in tumor cells (78). Further investigations confirmed that HGF upregulation and c-Met/AKT pathway activation triggered sorafenib resistance induced by exosomes derived from HCC cells (MHCC-97L and MHCC-97H), indicating that HGF/c-Met might be a

possible target for decreasing sorafenib resistance of HCC cells (79).

6. Exosomal miRNAs and proteins in gastric cancer

Gastric cancer (GC), a malignant tumor of the digestive system, is the second leading cause of cancer-related death and the fourth most common cancer worldwide (80). Although its incidence and mortality have appreciably decreased globally over recent decades, the mortality of GC is still relatively high in Asia (81).

Serving as biomarkers. Recent research suggested that serum exosomal miR-19b-3p and miR-106a-5 could be potential biomarkers for the early diagnosis of GC (82). Additionally, Tokuhisa *et al* (83) assessed exosomal miRNA profiles in peritoneal fluid and found that miR-21 and miR-1225-5p might be prognostic biomarkers for peritoneal recurrence after curative GC resection. miR-10b-5p, miR-195-5p, miR-20a-3p and miR-296-5p were significantly upregulated in serum exosomes derived from patients with GC and were able to discriminate GC patients from healthy controls (84).

Promoting metastasis. miR-214, miR-221 and miR-222 are commonly upregulated in gastric cancer tissue-derived mesenchymal stem cells (GC-MSCs) and tumor tissues; moreover, GC-MSC-derived exosomes deliver miR-221 to HGC-27 cells and promote the proliferation and migration (85). The serum exosomes of GC patients transport EGFR to liver cells, and EGFR activates HGF by suppressing miR-26a/b, stimulating the development of a liver-like microenvironment that promotes gastric cancer liver metastasis (86). In later studies, proliferation and Matrigel invasion of gastric cancer cells in the presence of exosomes derived from gastric cancer cells (SGC-7901) with either high (SGC/wt) or low (SGC/kd) CD97 expression were investigated, and the results indicated that CD97 promoted gastric cancer cell proliferation and invasion through exosome-mediated activation of the MAPK signaling pathway (87,88).

Regulating the immune response. Compared with exosomes derived from the untreated malignant ascites of GC patients, exosomes derived from heat-treated malignant ascites

Table III. Exosomal miRNAs in the top three mortality cancer types.

A, Lung cancer							
Author, year	miRNAs	Study design	Sample	Clinical significance	Approach	Performance	(Refs.)
Rabinowits <i>et al.</i> , 2009	miR-17-p/21/106a/146/155/191/192/203/205/210/212/214	Case-control	Human plasma	Diagnostic biomarkers for NSCLC	Microarray	Increase	(51)
Munagala <i>et al.</i> , 2016	miR-21/155	Animal model Cell model	Athymic nude mice H1299, Beas-2b	Possible prognostic markers for lung cancer recurrence	Microarray, qPCR	Increase	(52)
Liu <i>et al.</i> , 2017	miR-23b-3p/10b-5p/21-5p	Case-control	Human plasma	Independent non-invasive prognostic markers for NSCLC	qPCR	Increase	(53)
Liu <i>et al.</i> , 2016	miR-21	Patients Cell model	Human serum CSE-transformed HBE cells	Promoting CSE-induced angiogenesis and malignant transformation of HBE cells	qPCR	Increase	(58)
Hsu <i>et al.</i> , 2017	miR-23a	Patients Cell model	Human serum Hypoxic CL1-5	Stimulating the angiogenesis, intravasation and extravasation in lung cancer	qPCR	Increase	(59)
Fabbri <i>et al.</i> , 2012	miR-21/29a	Cell model, Animal model	A549, SK-MES WT B6 mice B6 TLR7-/-mice	Triggering tumour growth and metastasis	qPCR	Increase	(60)
Qin <i>et al.</i> , 2017	miR-100-5p	Cell model	A549/DDP	Altering the recipient lung cancer cells' resistance to DDP	Microarray, qPCR	Decrease	(61)
Yuwen <i>et al.</i> , 2017	miR-146a-5p	Patients Cell model	Human serum A549/DDP	Reversing the resistance of A549/DDP	qPCR	Increase	(62)
B, Liver cancer							
Author, year	miRNAs	Study design	Sample	Clinical significance	Approach	Performance	(Refs.)
Sohn <i>et al.</i> , 2015	miR-18a/221/222/224	Case-control	Human serum	Discriminating HCC from LC or CHB	qPCR	Increase	(67)
Sohn <i>et al.</i> , 2015	miR-101/106b/122/195	Case-control	Human serum	Discriminating HCC from CHB	qPCR	Decrease	(67)
Wang <i>et al.</i> , 2014; Liu <i>et al.</i> , 2017	miR-21/125b	Case-control	Human serum	Discriminating HCC from CHB or healthy controls	qPCR	Increase	(68,69)
Wei <i>et al.</i> , 2015	Vps4A-related miRNAs	Cell model	SMMC-7721, Hep3B, Huh-7	Regulating PI3K/AKT signaling pathway and promoting proliferation, invasion and metastasis of HCC cells	RNA sequencing	Increase	(71)
Lou <i>et al.</i> , 2015	miR-122	Cell model	AMSC	Enhancing the effect 5-FU or sorafenib on HCC cells	qPCR	Increase	(77)

Table III. Continued.

C, Gastric cancer							
Author, year	miRNAs	Study design	Sample	Clinical significance	Approach	Performance	(Refs.)
Wang <i>et al</i> , 2017	miR-19b-3p/ 106a-5	Case-control	Human serum	Potential biomarkers for the early diagnosis of GC	qPCR	Increase	(82)
Tokuhiya, <i>et al</i> , 2015	miR-21/ 1225-5p	Patients Cell model	Peritoneum lavage fluid, OCUM-2M OCUM-2MD3	Prognostic biomarkers for peritoneal recurrence after curative GC resection	Microarray, qPCR	Increase	(83)
Huang <i>et al</i> , 2017	miR-10b-5p/ miR-195-5p/ miR-20a-3p/ miR-296-5p	Case-control	Human serum	Discriminating GC patients from healthy controls	qPCR	Increase	(84)
Wang <i>et al</i> , 2014	miR-221	Patients Cell model Animal model	Human tissue GC-MSCs BALB/cnu/nu nude mice	Promoting HGC-27 cells proliferation and migration	Microarray, qPCR	Increase	(85)
Zheng <i>et al</i> , 2017	miR-21	Cell model Animal model	M2 macrophages athymic C57-nudemice	Conferring DDP resistance in GC cells	Microarray, qPCR	Increase	(92)
Wang <i>et al</i> , 2018	Anti-miR-214	Cell model Animal model	SGC7901, SGC7901/DDP BALB/c-nude mice	Reversing the resistance of GC cells to DDP	qPCR	Increase	(93)

NSCLC, non-small-cell lung cancer; CSE-transformed HBE cells, cigarette smoke extrac-transformed human bronchial epithelial cells. Hypoxic lung cancer cell, hypoxic CL1-5; DDP, cisplatin; HCC, hepatocellular carcinoma; LC, liver cirrhosis; CHB, chronic hepatitis B; AMSC, adipose tissue mesenchymal stem cell; 5-FU, 5-fluorouracil; GC, gastric cancer; GC-MSCs, gastric cancer tissue-derived mesenchymal stem cells; qPCR, quantitative polymerase chain reaction.

contained higher concentrations of the heat shock proteins Hsp70 and Hsp60, which might play an important role in inducing a tumor-specific cytotoxic T lymphocyte (CTL) response *in vitro* and are involved in the promotion of dendritic cell (DC) maturation (89). Additionally, HSPs have been identified as damage-associated molecular patterns (DAMPs), a class of self-danger signals released by stressed cells that elicited immune responses. Mechanistically, HSPs respond to the innate immune system both directly with inflammation and indirectly by recruiting reinforcements (90). However, there is some evidence showing that HSPs have a dampening effect on the immune system under physiological conditions, indicating that HSPs are actually DAMPERs, a class of molecules that reduces the activity of the innate immune system (91).

Mediating DDP resistance. The level of miR-21 in exosomes derived from tumor-associated macrophages (M2 macrophages) has been shown to be increased, and exosomal miR-21 can be directly transferred from tumor-associated macrophages to gastric cancer cells, conferring DDP resistance to gastric cancer cells by downregulating PTEN and activating signaling

through the PI3K/AKT pathway (92). However, exosome-delivered anti-miR-214 was able to reverse the resistance of gastric cancer cells to DDP, leading to suppressed migration *in vitro*, inhibited tumor growth *in vivo*, and increased cellular apoptosis (93). Additionally, MSC-derived exosomes significantly induced gastric cancer cell resistance to 5-FU both *in vivo* and *ex vivo* by activating the calmodulin-dependent protein kinase (CaM-K)/Raf/MEK/ERK pathway (94).

7. Conclusion and future studies

Exosomes have established a role in cancer biology, immunology, drug sensitivity and clinical diagnosis. In particular, exosomal miRNAs and proteins play important roles in cancers with high mortality rates (lung cancer, liver cancer and gastric cancer) (Tables III and IV).

On one hand, existing data indicate that the packaging of miRNAs into exosomes is a selective process and that the levels of specific exosomal miRNAs and proteins are changed in exosomes upon tumorigenesis. For these reasons, exosomal miRNAs and proteins can be served as a class of

Table IV. Exosomal proteins in the top three mortality cancer types.

A, Lung cancer							
Author, year	Protein	Study design	Sample	Clinical significance	Approach	Performance	(Refs.)
Yamashita <i>et al</i> , 2017	EGFR	Case-control	Human plasma	Potential diagnostic biomarker for characterization of lung cancer	ELISA	Increase	(54)
Sandfeld-Paulsen <i>et al</i> , 2016	NY-ESO-1	Case-control	Human plasma	A strongly prognostic markers for poor survival of NSCLC	Microarray	Increase	(55)
Ueda <i>et al</i> , 2014	CD91	Case-control	Human serum	Diagnostic markers for ADC	ELISA Mass spectrometry	Increase	(56)
B, Liver cancer							
Author, year	Protein	Study design	Sample	Clinical significance	Approach	Performance	(Refs.)
He <i>et al</i> , 2015	CAV1/CAV2/S100A4	Cell model	HKCI-C3, HKCI-8 MHCC97L	Enhancing the invasive and migratory abilities of non-motile MIHA cells	Western blot Mass spectrometry	Increase	(75)
Qu <i>et al</i> , 2016	HGF	Cell model Animal model	MHCC-97L, MHCC-97H BALB/c nu/nu mice	Improving sorafenib resistance of HCC cells	ELISA Western blot	Increase	(79)
C, Gastric cancer							
Author, year	Protein	Study design	Sample	Clinical significance	Approach	Performance	(Refs.)
Zhang <i>et al</i> , 2017	EGFR	Patients Animal model Cell model	Human serum/tissue BALB/ c-nu nude mice SGC7901	Promoting GC liver metastasis	ELISA Western blot	Increase	(86)
Li <i>et al</i> , 2015; Liu <i>et al</i> , 2016	CD97	Cell model	SGC-7901	Promoting GC cells proliferation and invasion	Western blot	Increase	(87,88)
Zhong <i>et al</i> , 2011	Hsp70, Hsp60	Patients	Heat-treated malignant ascites	Inducing a CTL response <i>in vitro</i> and involving in the promotion of DC maturation	Western blot	Increase	(89)
NSCLC, non-small-cell lung cancer; ADC, lung adenocarcinoma; MIHA, motile immortalized hepatocyte; HCC, hepatocellular carcinoma; CTL, cytotoxic T lymphocyte; DC, dendritic cell.							

novel biomarkers for clinical applications in high-mortality cancers. Moreover, the specificity, sensitivity and diagnostic value of exosomal miRNAs and proteins may be superior to

that of traditional tumor markers. On the other hand, exosomal miRNAs and proteins are delivered between tumor cells to transmit information and modulate signaling pathways. Taken

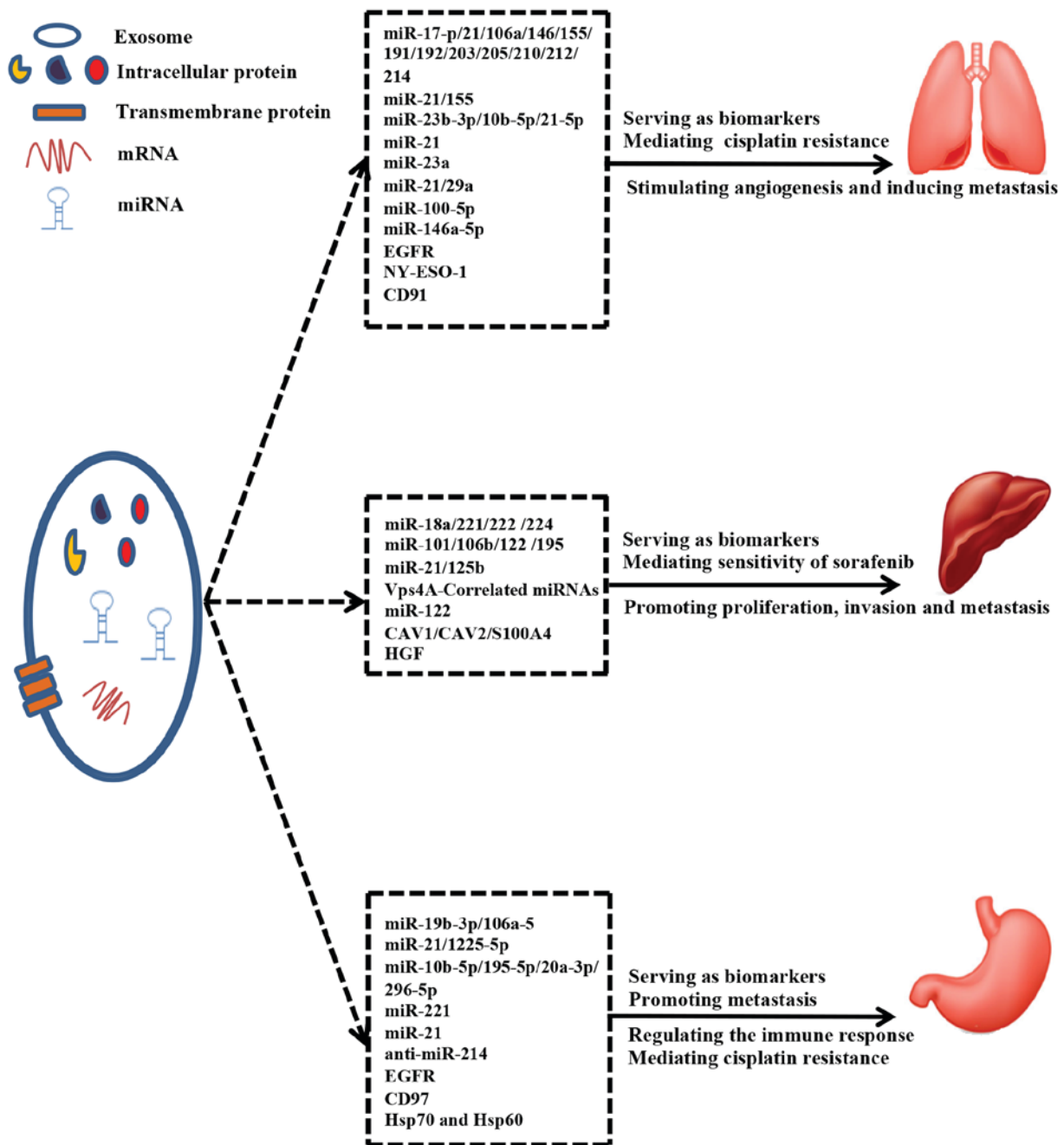


Figure 3. Exosomal miRNAs and proteins play vital roles in high-mortality cancers. Exosomal miRNAs and proteins can be used as diagnostic or prognostic biomarkers, promote tumor progression and metastasis, and simultaneously regulate immune response and tumor cells' sensitivity to chemotherapy drugs.

together, exosomal miRNAs and proteins perform the essential function of promoting tumor progression and metastasis as well as mediating the immune response and sensitivity of tumor cells to chemotherapy drugs (Fig. 3).

In the future, more robust techniques, such as RNA-Seq and mass spectrometry, can be used for the detection, characterization and discovery of exosomal miRNAs and proteins. Moreover, exosomes could efficiently deliver chemotherapeutic agents to cells and tissues. Therefore, these bioengineered, drug-loaded exosomes can serve as promising exosome mimetics for effective chemotherapeutic agent delivery, which will be applied for the target treatment of malignant tumors. Currently, the majority of research on chemotherapy resistance and exosomal microRNAs focuses on cisplatin, and little is known about

other drugs. To identify more sensitive and specific exosomal miRNAs and proteins to guide personal chemotherapy selection, future studies should further elucidate the role and underlying mechanism of exosomal miRNAs and proteins in more diverse cancers with more chemotherapy drugs.

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Availability of data and materials

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

LML was a major contributor in writing the manuscript. HL and XHL were responsible for the collection of the relevant literatures. HBH and SML revised the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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