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REVIEW

The biological functions and clinical applications of exosomes in lung cancer

Rui Chen[1] · Xin Xu[1] · Zijun Qian[1] · Congcong Zhang[1] · Yongjie Niu[1] · Zhixian Wang[1] · Jianli Sun[3] · Xiao Zhang[2] · Yongchun Yu[1,2]

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Abstract
Lung cancer remains the leading cause of cancer-related death worldwide, and the high incidence rates are worrisome. Exosomes are a class of extracellular vesicles secreted by most cells, including RNAs, proteins and lipids. Exosomes can mediate cell-to-cell communication in both physiologic and pathologic processes. Accumulated evidences show that cancer-derived exosomes aid in the recruitment and reprogramming of constituents correlated with tumor microenvironment. Furthermore, exosome-based clinical trials have been completed in advanced lung cancer patients. In this review, we discuss the roles of exosomes in a lung cancer microenvironment, such as its participation in lung cancer initiation, progression and metastasis as well as being involved in angiogenesis, epithelial–mesenchymal transition (EMT), immune escape, and drug resistance. In addition, we focus on the potential of exosomes as diagnostic and prognostic biomarkers in lung cancer, as well as the challenges faced by and advantages of exosomes as drug delivery vehicles and in exosome-based immunotherapy.

Keywords Lung cancer · Exosomes · Biomarkers · Immunotherapy · Drug delivery vehicles

Introduction

Lung cancer remains the leading cause of cancer-related death worldwide with 1.8 million deaths predicted in 2018, accounting for almost 1 in 5 (18.4%) cancer deaths [1]. Lung cancer is divided into small-cell lung cancer (SCLC, about 15%) and non-small-cell lung cancer (NSCLC, about 85%) containing adenocarcinoma and squamous cell carcinoma [2]. Of importance, great progress has been made in the treatment of NSCLC, such as epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) [3–7], anaplastic lymphoma kinase (ALK) inhibitors [8], and bevacizumab [9], as well as immune checkpoints inhibitors [10–17]. However, only a small proportion of patients benefit from these drugs. In recent years, the biofunctional and biochemical nature of exosomes have been extensively studied, which can shed new light on the molecule mechanism of lung cancer initiation and progression, and contribute to developing new diagnostic and therapeutic strategies for lung cancer patients.

Exosomes are a class of extracellular vesicles (EVs) of endocytic origin with a diameter of 30–100 nm and a density in sucrose gradients of 1.13–1.19 g/ml [18–20]. Vesicles released during the maturation of sheep reticulocytes were first defined as exosomes by Johnstone et al. [21].

Exosomes are membrane-bound phospholipid vesicles, including proteins, nucleic acids (mRNA and noncoding RNAs) and lipids [19, 22]. Exosomes are secreted by most cell types, in particular tumor cells; tumor-derived exosomes play an important role in the communication between tumor cells and their microenvironment, contributing to creating a favorable soil for tumor progression [23]. Noteworthy, exosomes have been isolated in most biological fluids, such as serum and plasma, saliva, urine, breast milk, semen,

✉ Jianli Sun
1721679167@qq.com

✉ Xiao Zhang
386229418@qq.com

✉ Yongchun Yu
yuyongchun1255@126.com

[1] Shanghai Municipal Hospital of Traditional Chinese Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai 200071, China

[2] Shanghai Chest Hospital, Shanghai Jiao Tong University, Shanghai 200030, China

[3] Department of Oncology, Longhua Hospital Shanghai University of Traditional Chinese Medicine, Shanghai 200032, China

amniotic fluid, cerebrospinal fluid, pleural effusions, bronchial lavage, ascites fluid, synovial fluid and bile [24–27]. Moreover, exosomes provided a protective vesicle for transported small RNAs against degradation of RNase [28]. These special features make exosome an ideal specimen for liquid biopsy. In addition, as the therapeutic potential of exosome was explored, exosome-based phase I and II clinical trials have been completed in advanced lung cancer patients, with a favorable safety profile [29, 30]. On the other hand, exosomes have recently come into focus as drug delivery vehicles that can deliver chemotherapy drugs and biologics for lung cancer therapy [31–33].

Here, we describe how exosomes are formed and released into extracellular space, and discuss the advantages and limitations of some techniques used for exosomes isolation and purification. We also summarize the most recent studies on exosomes that participated in the reprogramming of the lung cancer microenvironment. Lastly, we focus on the roles of exosomes as potential biomarkers and drug delivery vehicles, as well as in exosome-based immunotherapy.

Formation, release and uptake of exosomes

Extracellular vesicles are a heterogeneous population of membrane vesicles that based on their biogenesis are classified into three distinct classes: exosomes, microvesicles and apoptotic bodies (Fig. 1) [34–39]. Exosome is initiated when membrane proteins are endocytosed via inward budding of the plasma membrane to form the early endosome, then, early endosomes mature into late endosomes, which are also known as multivesicular bodies (MVBs) containing numerous intraluminal vesicles (ILVs) formed by the invagination of the endosomal membrane [35]. During invagination, cytosolic and membrane proteins, lipids, and RNAs are incorporated into the ILVs [40, 41]. MVBs can fuse with lysosomes, resulting in the degradation of cargos carried by vesicles [40, 42]. Alternatively, MVBs may fuse with the plasma membrane, releasing exosomes to the extracellular space [43].

The endosomal sorting complex required for transport (ESCRT) and associated proteins (Clathrin, TSG101 and ALIX) are involved in ubiquitinated proteins sorting and ILVs formation [44–49]. However, the cargos sorting and ILVs formation are not solely dependent on ESCRT complexes, but required the sphingolipid ceramide [50]. In addition, tetraspanins are also involved in the biogenesis of exosomes, such as CD9, CD63, CD81 and CD82 enriched in exosomes from various types of cells, which are able to form oligomeric complexes and interact with integrins and MHC molecules [51, 52]. The Rab family of small GTPases, for example, Rab5 is required for the biogenesis of the early endosomes, late endosomes and lysosomes [53, 54], Rab7 regulate the transfer of cargos from the MVBs to the lysosomes [55], Rab35, Rab27a and Rab27b are involved in MVBs fusion with plasma membrane and exosomes secretion [56, 57]. However, the mechanisms that control the sorting of specific RNA species to exosomes are yet to be shown. Kosaka et al. showed that miRNAs can be loaded into exosomes and released through a ceramide-dependent pathway but not ESCRT machinery [58]. Villarroya-Beltri et al. reported that the protein heterogeneous nuclear ribonucleoprotein A2B1 (hnRNPA2B1) controlled miRNAs sorting to exosomes through binding to a specific motif of miRNA [59]. Squadrito et al. demonstrated that miRNA loading into exosomes was regulated by cell activation-dependent changes in miRNA target levels in the parental cells [60].

Owing to their endosomal origin, nearly all exosomes contain proteins involved in MVBs biogenesis (clathrin, TSG101 and ALIX), intracellular membrane transport and fusion (GTPases, flotillins and annexins), and tetraspanins (CD9 and CD63), which are used as surface markers for exosomes identification [61]. Exosomes contain heat shock proteins (Hsp70 and Hsp90) involved in antigen presentation and package peptides into MHC molecules [62]. Exosomes also contain integrins that possibly address exosomes to recipient cells [18]. In addition, exosomes are enriched in lipid rafts, such as cholesterol, ceramide and sphingolipids, which show difference in the distribution of membrane lipids from their parental cells [63].

Then, exosomes get to the recipient cells, after that they may bind to the surface receptors and induce intracellular signaling [64, 65]. Exosomes can be taken up by recipient cells through clathrin-dependent and clathrin-independent endocytosis, such as pinocytosis, phagocytosis and macropinocytosis [66–69]. Additionally, exosomes also can be internalized by caveolae and lipid raft-mediated endocytosis [41]. On the other hand, exosomes could directly fuse with the plasma membrane and release their contents into the cytoplasm [70].

Isolation and purification of exosomes

Exosomes isolation and purification from cell culture supernatants and biological fluids are essential to biomedical investigation and clinical application. In general, the isolation methods of exosomes are mainly based on the physical (such as size, density and molecular weight), chemical (surface charge) and biological (specific markers) properties of exosomes. Ultracentrifugation is the most widely used conventional protocol for exosomes isolation, which involves a series of centrifugations at increasing speed to remove dead cells and large apoptotic debris, followed by a final high-speed ultracentrifugation

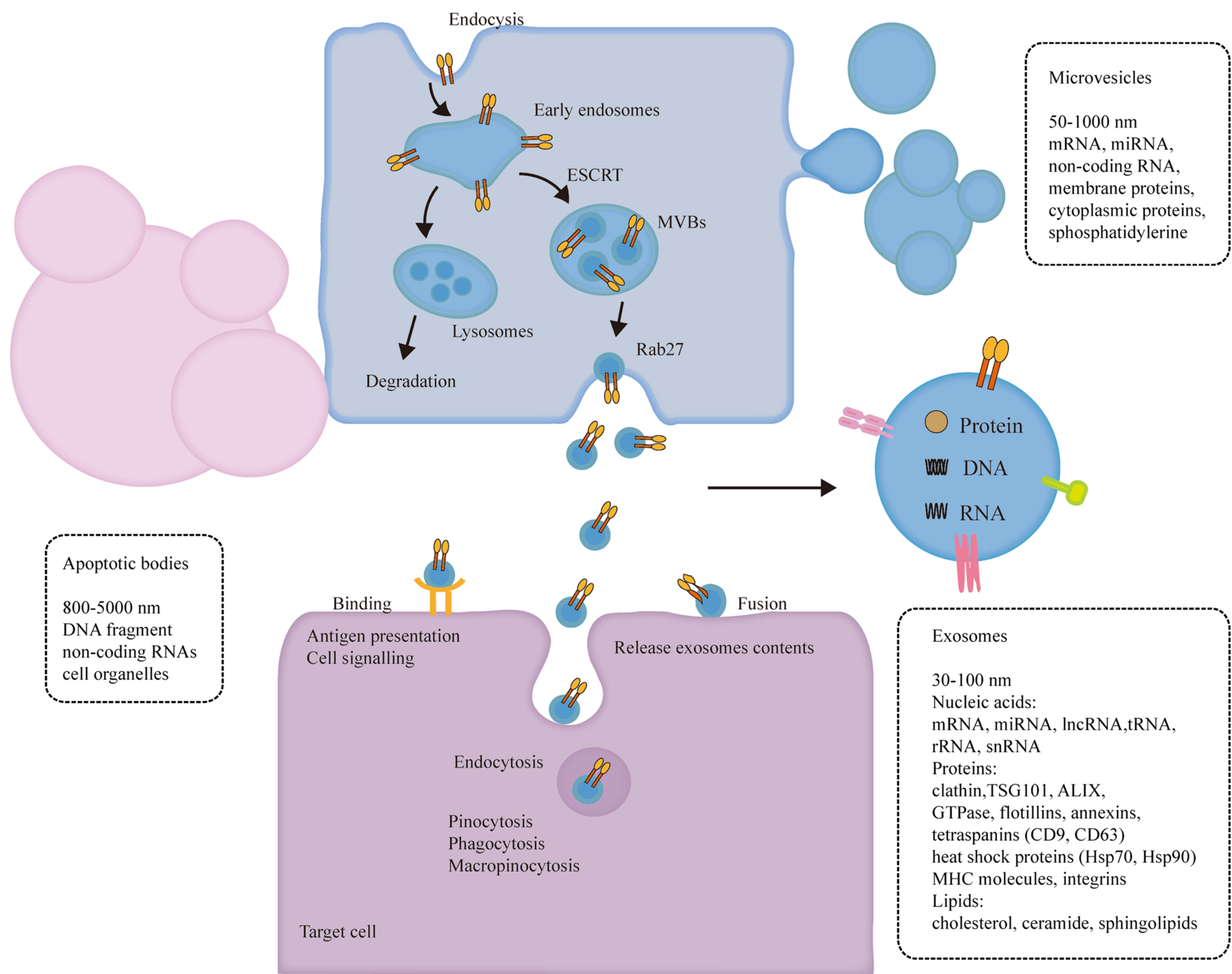

Fig. 1 Formation, release and uptake of exosomes. Extracellular vesicles are classified into three distinct classes: exosomes, microvesicles and apoptotic bodies. Apoptotic bodies are released from the plasma membrane during cell undergoing programmed cell death [32]. Microvesicles are shed directly outwards budding from the plasma membrane [33, 34]. Exosome formation starts when membrane proteins are endocytosed via inward budding of the plasma membrane to form the early endosome and exosomes are formed as intraluminal vesicles (ILVs) by the invagination of the endosomal membrane. Finally, exosomes are released by fusion of the MVBs with the plasma membrane. Several machineries are involved in sorting of cargos into ILVs and formation of ILVs. When exosomes bind to the recipient cells, they may remain bound to the surface and induce intracellular signaling, or they directly fuse with the plasma membrane and release their contents into the cytoplasm. Additionally, exosomes can be internalized by clathrin-independent or clathrin-dependent endocytosis, including pinocytosis, phagocytosis and macropinocytosis

(100,000*g* for 70 min) to precipitate exosomes [71]. Nevertheless, the method requires a lot of time and costly instruments (ultracentrifuge), and precipitated exosomes may contain aggregated proteins [72]. Owing to exosomes have a floatation density of 1.13–1.19 g/ml on sucrose gradients [18], based on extracellular vesicles different floatation densities, using sucrose-gradient centrifugation helps to further separate different vesicular density [73–75], therefore, this method is thought to precipitate exosomes at a higher purity. Recently, several commercial kits, for example, ExoQuick™ (System Biosciences) that rely on polymer co-precipitation have been widely used to isolate exosomes [76]. The precipitated exosomes via ExoQuick™ could be easily and quickly isolated with low centrifugal forces, whereas this approach is likely to co-isolate heterogeneous polymeric particles and lack specificity [77]. Exosomes express abundant membrane proteins, such as MHC I and II molecules, costimulatory molecules, and adhesion molecules that can be used as markers for exosomes immune isolation. Clayton et al. utilized magnetic beads, coated with monoclonal antibodies specific to HLA DR, DQ and DP for the particular isolation of exosomes from cell-free supernatants, which is a rapid protocol for the isolation of antigen-presenting

cells (APCs) exosomes [78]. In particular, ultrafiltration is suitable for purifying exosomes from large volumes (> 1 L) of conditioned medium [71]. Lamparski et al. combined ultrafiltration and ultracentrifugation for the purification of clinical-grade exosomes derived from APCs [79]. On the other hand, size-exclusion purification is comparable to density gradient isolation of exosomes [80]. Boing et al. used size-exclusion chromatography (SEC) to isolate extracellular vesicles from human plasma and they investigated that SEC purified extracellular vesicles with diameter larger than 70 nm from human platelet-free supernatant of platelet concentrates, moreover, the vesicle pellets less than 5% of HDL and less than 1% of protein [81]. In addition, field flow fractionation [82] and microfluidic chip [83] are also developed to elevate purification efficiency. Of note, fetal bovine serum (FBS) also contains exosomes and influences cultured cell phenotypes, thus, conditioned medium was replaced by FBS eliminated of exosomes via 18 h ultracentrifugation before the exosomes were extracted [84]. After isolation and purification of exosomes from cell culture supernatant or other biological fluids, the vesicles size distribution, morphologies and surface markers were further analyzed to verify that they are exosomes, and the isolation and detection technologies of exosomes are summarized in Table 1.

The role of exosomes in the lung cancer microenvironment

Exosomes contain RNAs, proteins, and lipids, all of which affect exosomes functions and reflect cell characteristics. The major nucleic acids of exosomes are RNAs that contain microRNA (miRNA), tRNA, and long noncoding RNA (lncRNA), as well as largely fragmented mRNAs [95, 96]. Exosomes transfer RNAs to recipient cell, mRNA may be translated into proteins, and miRNAs can modulate the translation of target mRNA in host cells [97]. In addition, exosomal proteins can be also transported to recipient cells and trigger strong cellular responses [98]. Exosomes can serve as a novel cellular communication by transferring their contents to target cells in a lung cancer microenvironment, thereby regulating lung cancer cells progression (Fig. 2 and Table 2).

Exosomes in lung cancer angiogenesis

Tumor growth and metastasis require new blood vessels to supplement oxygen and nutrients, which activate vasculogenesis and angiogenesis, and consequently the sprouting of blood vessels from the neighboring tissues into the tumor [99]. Zhuang et al. found that tumor-derived miR-9 was transferred into endothelial cells via microvesicles and

Table 1 Isolation and detection technologies for exosomes

Contents	Platform	References
Isolation and purification methods	Ultracentrifugation	[71, 72]
	Sucrose-gradient centrifugation	[73–75]
	Polymer co-precipitation	[76, 77]
	Antibody-coated magnetic beads	[78]
	Ultrafiltration	[71, 79]
	Size-exclusion chromatography	[81]
	Field flow fractionation	[82]
	Microfluidic chip	[83]
Analysis of morphology and size distribution	Transmission electron microscopy (TEM)	[72]
	Scanning electron microscopy (SEM)	[85]
	Cryo-electron microscopy (cryo-EM)	[86]
	Atomic force microscopy (AFM)	[87]
Analysis of concentration and size distribution	Nanoparticle tracking analysis (NTA)	[88]
	Dynamic light scattering (DLS)	[89]
	Tunable resistive pulse sensing (TRPS)	[90]
Protein quantification and characterization	Western blotting	[80]
	Enzyme-linked immunosorbent assay (ELISA)	[91]
	Mass spectrometry	[92]
Nucleic acid amplification and sequencing	Polymerase chain reaction (PCR)	[93]
	Next-generation sequencing (NGS)	[94]

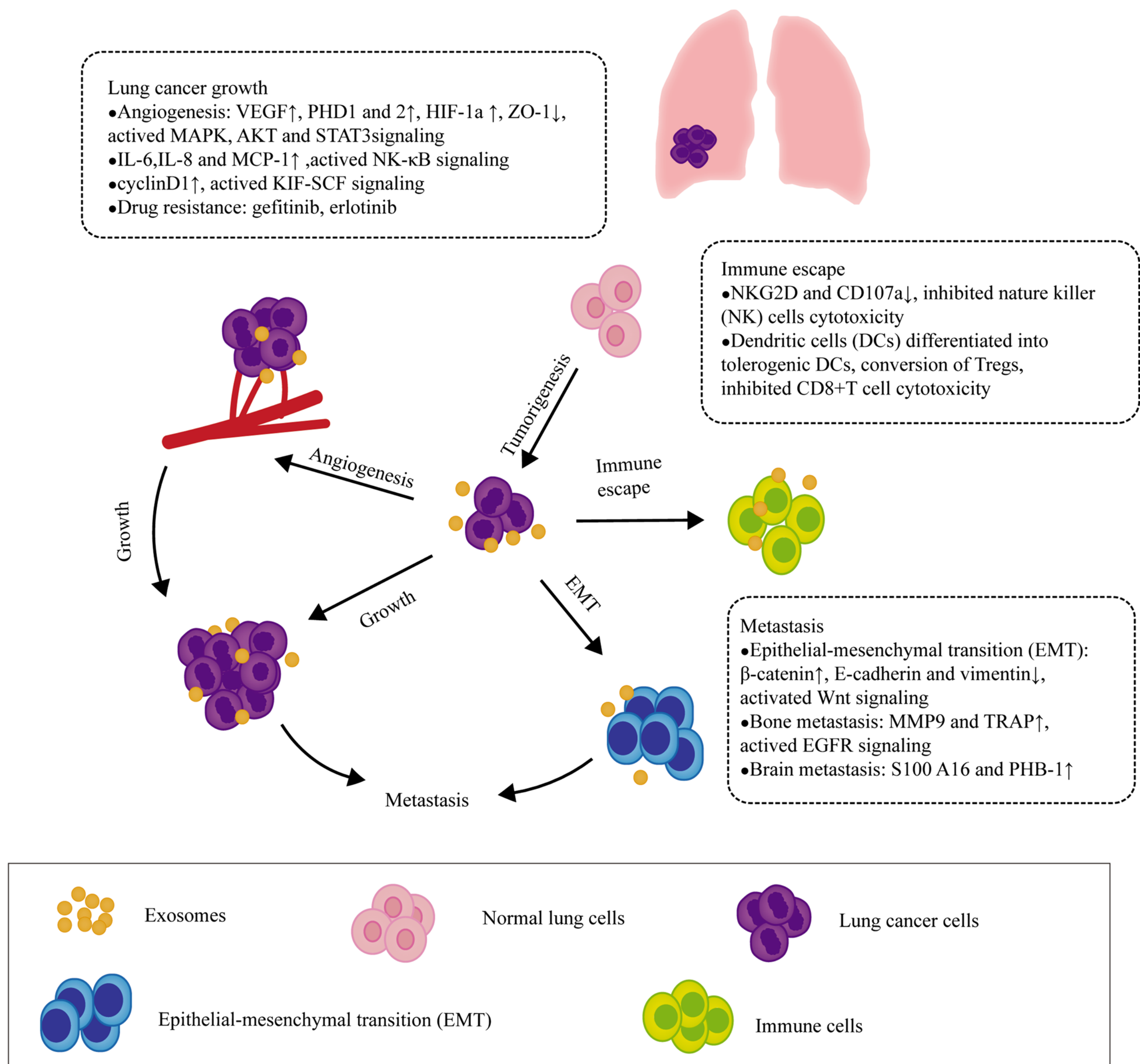

Fig. 2 The role of exosomes in a lung cancer microenvironment. Exosomes mediate communication between lung cancer cells and their surrounding microenvironment through transferring their biologically active cargos as well as participate in lung cancer initiation, progression and metastasis involved in angiogenesis, epithelial–mesenchymal transition (EMT), immune escape and drug resistance

that miR-9 effectively decreased the suppressor of cytokine signaling 5 (SOCS5) level, and induced the activation of JAK–STAT pathway, ultimately promoting endothelial cell migration and tumor angiogenesis [100]. Exosomes secreted by A549 cells harboring activated EGFR can be internalized by cocultured endothelial cells, which activated MAPK and AKT signaling, and induced expression of vascular endothelial growth factor (VEGF) in endothelial cells, followed by autocrine activation of VEGF receptor-2 (VEGFR-2), facilitating tumor angiogenesis [101]. Under hypoxic conditions, miR-23a was significantly increased in exosomes from lung cancer cells, and exosomes transferred miR-23a to endothelial cells; miR-23a directly inhibited its target prolyl hydroxylase 1 and 2 (PHD1 and 2) expression, resulting in the accumulation of hypoxia-inducible factor-1 α (HIF-1 α) in endothelial cells. Consequently, exosomes derived from hypoxic lung cancer cells increased endothelial permeability and cancer cells transendothelial migration in vitro, enhanced neovascularization and tumor growth in vivo [102]. Liu et al. found that smoking induced an increase in the serum miR-21 level, and exosomes isolated from cigarette smoke extract (CSE)-transformed human

Table 2 Exosomal cargos detected in lung cancer and clinical relevance

Exosomal Cargos	Parental cells	Recipient cells	Target	Biological/clinical relevance	Reference
(1) miRNA					
miR-9	H1299 cells	Endothelial cells	SOCS5	Promoted endothelial cell migration and tumour angiogenesis	[100]
miR-23a	CL1-5 cells	Endothelial cells	PHD1 PHD2	Increased endothelial permeability and cancer cells transendothelial migration	[102]
miR-21	CSE-transformed HBECs	Normal HBECs	VEGF	Facilitated angiogenesis	[103]
miR-210	A549L cells	HUVECs	TIMP-1	Increased vascularization in A549L-derived tumour xenografts	[150]
miR-23a	(TGF)-β-treated A549 cells	A549 cells	TCF4/β-catenin	EMT	[105]
miR-21 miR-29a	A549 cells	Immune cells	TLRs	Led to tumor growth and metastasis	[113]
miR-142-3p	H1437 H2073	Endothelial and fibroblast cells	TGFβR1(endothelial cells)	Promoted angiogenesis in endothelial cells; facilitated the CAFs transformation in lung fibroblast cells	[115]
miR-223	Platelets	A549 cells	EPB41L3	Promoted A549 cells invasion	[123]
miR-21	A549 cells	Osteoclast progenitor cells	PDCD4	Promoted osteoclastogenesis	[127]
miR-21	H827R cells	HCC827 cells		Led to gefitinib resistance in HCC827 cells	[129]
miR-214	PC-9GR cells	PC9cells		Mediated gefitinib resistance	[132]
(2) Protein					
EGFR	A549 cells	Endothelial cells	VEGF	Facilitated tumor angiogenesis	[101]
Vimentin	PC14HM cells	HBECs		EMT	[110]
HSP70	A549 cells	MSCs	TLR2	Induced a pro-inflammatory phenotype in MSCs	[111]
KIT	Mast cells	A549 cells	cyclin D1	Accelerated the proliferation of A549 cells	[112]
CD41	Platelets	A549 cells	cyclin D2	Facilitated lung cancer cells proliferation	[114]
EGFR	Lung cancer cells	DCs		Suppressed tumor specific CD8 + T cells	[120]
MMP3	Adipocytes	3LL cells		Promoted 3LL cells invasion	[125]
AREG	CRL-2868	Osteoclasts	MMP9 TRAP	Triggered osteolytic bone metastasis	[126]
S100A16	SCLC cells	HBMECs	PHB-1	Facilitated SCLC cells brain metastasis	[128]
(3) lncRNA					
lnc-MMP2-2	(TGF)-β-treated A549 cells	A549 cells	MMP2	Regulated vascular permeability and lung cancer invasion	[104]
RP11-838N2.4	HCC4006/R HCC827/R	HCC4006 HCC827	FOXO1	Reduced resistance to erlotinib treatment	[130]
lncRNA H19	HCC4006/R HCC827/R	HCC4006 HCC827		Induced resistance to gefitinib treatment	[133]

SOCS5 suppressor of cytokine signaling 5, *PHD1 and 2* prolyl hydroxylase 1 and 2, *HUVECs* human umbilical vein endothelial cells, *CSE* cigarette smoke extract, *(MMP)2-2* lnc-matrix metalloproteinase, *TIMP-1* tissue inhibitor of metalloproteinases-1, *HBECs* human bronchial epithelial cells, *MSCs* mesenchymal stem cells, *TLR2* Toll-like receptor 2, *Lnc-MMP-2-2* lnc-matrix metalloproteinase, *TLRs* Toll-like receptors, *CD41* glycoprotein IIb/IIIa, *CAFs* cancer-associated fibroblast, *DCs* dendritic cells, *EPB41L3* the 3′UTR of erythrocyte membrane protein band 4.1-like 3, *3LL* Lewis lung cancer cells, *AREG EGFR* binds amphiregulin, *MMP9* matrix metalloproteinases 9, *TRAP* tartrate-resistant acid phosphatase, *HBMECs* human brain microvascular endothelial cells, *PHB-1* prohibitin-1, *FOXO1* forkhead box protein O1

bronchial epithelial (HBE) cells also showed a high level of miR-21. Transformed HBE cells delivered miR-21 into normal HBE cells by exosomes, which activated IL-6/STAT3 signaling and enhanced the expression of VEGF in recipient cells, facilitating angiogenesis in HBE cells [103]. Wu et al. screened a exosomal lncRNA and lnc-matrix metalloproteinase (MMP)2-2 was remarkably enriched in transforming growth factor (TGF)-β-treated A549 cells, and promoted

MMP2 expression. Coculture experiments revealed that TGF-β-mediated exosomes, as vectors of intercellular communication, regulated vascular permeability and lung cancer invasion [104]. Due to overexpression of tissue inhibitor of metalloproteinases-1 (TIMP-1) in lung cancer cells, miR-210 was up-regulated in exosomes. These exosomes facilitated tube formation activity in human umbilical vein endothelial cells (HUVECs), which was reflected in increased vascularization in A549L-derived tumor xenografts [105]. Together, these results support that exosomes as a novel pro-angiogenic mechanism can regulate vascular remodeling in tumor microenvironment by directional transfer of the angiogenic factors between tumor cells and endothelial cells, thereby promoting tumor angiogenesis.

Exosomes and epithelial–mesenchymal transition (EMT)

Epithelial–mesenchymal transition (EMT) is a highly conserved process confirmed by the loss of cell–cell adhesion and acquisition of the mesenchymal phenotype, which is in favor of tumor cells being migratory and invasive [106]. Interestingly, recent studies indicated that exosomes participated in the EMT process, during which exosomes transferred mesenchymal-associated information between the tumor cells and the microenvironment, and regulated signal transduction in recipient cells [107, 108]. Exosomes isolated from TGF-β1-induced mesenchymal cell showed that the expression of β-catenin increased and E-cadherin as well as vimentin decreased. Meanwhile, miR-23a was significantly up-related in secreted exosomes. Further investigation showed that exosome stimulated TCF4/β-catenin transcriptional activity and activated canonical Wnt signaling in A549 cells undergoing EMT [109]. Rahman MA et al. found exosomes derived from highly metastatic lung cancer PC14HM cells that express higher vimentin than those from non-metastatic lung cancer PC14 cells. When human bronchial epithelial cells (HBECs) were treated with PC14HM exosomes, vimentin expression was significantly up-related, inducing EMT in recipient HBECs [110]. These results suggest tumor-derived exosomes to be an important mediator of EMT, thereby transforming tumor cells to a more aggressive phenotype, though more studies need to be performed.

Exosomes regulate lung cancer growth

Emerging evidence indicate that exosomes play a bimodal role in cancer and they can program the local and systemic environment to help cancer growth and dissemination [23]. A549 cell-derived exosomes were internalized by mesenchymal stem cells (MSCs), which significantly stimulated IL-6, IL-8, and MCP-1 production and recruited macrophages, promoting tumor growth. It was further detected that Hsp70 on the surface of A549 cell-derived exosomes activated NF-κB signaling through Toll-like receptor 2 (TLR2), inducing a pro-inflammatory phenotype in MSCs [111]. Xiao et al. demonstrated mast cells like HMC-1-derived exosomes contain c-KIT mRNA, and HMC-1 exosomes transferred KIT protein to A549 cells, subsequently activating KIT-SCF signaling, which increased cyclin D1 expression and accelerated the proliferation of A549 cells [112]. Fabbri et al. identified that miR-21 and miR-29a were significantly up-regulated in exosomes isolated from A549 cells. Exosomal miR-21and miR-29a bound and activated Toll-like receptors (TLRs) in surrounding immune cells, leading to tumor growth and metastasis [113]. Platelet-derived exosomes delivered the glycoprotein IIb/IIIa (CD41) to the surface of lung cancer cells, and induced phosphorylation of MAPK p42/44 and AKT as well as the expression of cyclin D2 in lung cancer cells, facilitating lung cancer cells proliferation [114]. EVs transferred miR-142-3p from lung adenocarcinoma cells to both endothelial and fibroblast cells, which promoted angiogenesis by inhibition of TGFβR1 in endothelial cells. In additional, EV-associated miR-142-3p also facilitated the cancer-associated fibroblast (CAF) transformation but did not target TGFβR1 in lung fibroblast cells [115]. Taken together, these data indicate that exosomes can transfer miRNA from lung cancer cells to other non-cancer cells and regulate gene expression in the host cells through binding to their target mRNAs, moreover, exosomal proteins also directly activate signaling in the host cells, with resultant regulation growth and proliferation of lung cancer.

Exosomes and lung cancer immune escape

Tumor-derived exosomes carry immunosuppressive molecules, and transfer these cargos to immune cells and directly or indirectly suppress the functions of immune cells, thereby promoting tumor progression [116]. Chen et al. reported that exosomes from human melanoma, breast and lung cancer cells carry immunosuppressive PD-L1, and PD-L1 binds to PD-1 via its extracellular domain to inactivate T cells. The level of exosomal PD-L1 was up-regulated by interferon-γ (IFN-γ), which suppressed the function of CD8 T cells and facilitated tumor growth [117]. In addition, Poggio et al. also uncovered the mechanism that exosomal PD-L1 enables tumor cells to extend survival, and genetic blockade of exosomal PD-L1 can facilitate T-cell activity in the draining lymph node, thereby inducing systemic antitumor immunity and memory [118]. Berchem et al. reported that hypoxic tumor-derived microvesicles (TD-MVs) carried immunosuppressive molecules (TGF-β1 and miR-23a) that can inhibit natural killer (NK) cells cytotoxicity in vitro and in vivo

[119]. Huang et al. discovered that more than 80% exosomes isolated from the lung cancer biopsies contained EGFR, and dendritic cells (DCs) can capture these exosomes to differentiate into tolerogenic DCs, which could convert the naïve CD4+ T cells into tumor antigen-specific regulatory T cells (Tregs), thus suppressing the function of tumor specific CD8+ T cells [120]. Collectively, these data show that tumor-derived exosomes can rescue tumor cells by evading the surveillance of immune cells, which could represent a therapeutic target, contribute to developing immunotherapeutic approaches for cancer therapy.

Exosomes and lung cancer metastasis

Metastasis is a complex multistep process of tumor cells invasion, survival in blood vessels, attachment to and colonization of the host organs, during which exosomes act as functional mediators in cell–cell communication, influencing the various steps of the metastatic cascades [121]. Hoshino et al. have demonstrated that certain exosomal integrins are 'addressing' cancer cells to specific organs and predict organ-specific metastasis, among which exosomal integrins α6β4 and α6β1 were linked to lung metastasis, while exosomal integrin αvβ5 was associated with liver metastasis [122]. Liang et al. demonstrated that platelets from NSCLC patients expressed higher levels of miR-223 than those from healthy subjects. Incubation of A549 cells with platelet-secreted microvesicles suppressed the translation of 3′UTR of erythrocyte membrane protein band 4.1-like 3 (EPB41L3) in A549 cells, promoting A549 cells invasion [123]. Exosomes from pigment epithelium-derived factor (PEDF)-treated A549 cells significantly inhibited cells motility compared to control groups, and it was further found that PEDF decreased cancer-derived exosome-mediated metastatic activity through elevated thrombospondin 1(THBS1) expression and increased its contents in exosomes. Owing to THBS1 suppressed cytoskeletal remodeling and the migration and invasion of lung cancer cells, the presence of THBS1-containing exosomes in the pre-metastatic niche could prevent lung cancer progression [124]. Compared with lung cells, MMP3 proteins were increased in 3T3-L1 adipocytes. 3T3-L1 adipocyte-derived exosomes (3T3-A-EXO) transferred MMP3 to 3LL Lewis lung cancer cells, then, MMP3 induced MMP9 expression in 3LL cells and promoted 3LL cells invasion in vitro and in vivo [125].

In NSCLC, bone metastasis is the frequent complication. Taverna S et al. investigated that exosomes derived from NSCLC cells contain a high level of EGFR binds amphiregulin (AREG), which induce the activation of EGFR signaling in pre-osteoclasts that in turn up-regulates the expression of matrix metalloproteinases 9 (MMP9) and tartrate-resistant acid phosphatase (TRAP), well-known genes involved in osteoclasts differentiation, triggering osteolytic bone metastasis [126]. In addition, Xu et al. demonstrated that miR-21 overexpression in exosomes from A549 cells, and exosomal miR-21 was taken up by osteoclast progenitor cells, inhibited PDCD4 expression in recipient cells, thereby promoting osteoclastogenesis [127].

SCLC is a strong preference for early brain metastases, which lead to most SCLC-related deaths. SCLC cells cocultured with exosomes derived from human brain microvascular endothelial cells (HBMECs) showed that S100A16, a partner of the annexin family of proteins, was significantly up-regulated. Meanwhile, HBMECs exosomes induced translocation of S100A16 in the recipient SCLC cells from the cytoplasm to the nucleus, which triggered the expression of prohibitin (PHB)-1, a protein in the mitochondria inner membrane to maintain mitochondrial membrane potential, facilitating brain metastatic SCLC cells to escape death signals and survive in the brain [128].

Taken together, these findings indicate that tumor-derived exosomes serve as a crosstalk between cancer cells and their local and distant microenvironment, and are capable of mediating organ-specific metastasis, which provide support for Stephen Paget's "seed-and-soil" hypothesis.

Exosomes and EGFR-TKIs resistance

EGFR-TKIs, containing the first generation of gefitinib and erlotinib, are standard first-line therapy for NSCLC patients who harbor sensitivity EGFR mutations, yet, acquired resistance to EGFR-TKIs is inevitable. Jing et al. reported that gefitinib-resistant H827R cells transferred miR-21 to gefitinib-sensitive HCC827 cells by exosomes, which activated the AKT signaling in gefitinib-sensitive HCC827 cells, thereby leading to HCC827 cells to gefitinib resistance [129]. Zhang et al. identified lncRNA RP11-838N2.4 was increased in erlotinib-resistant cells compared to erlotinib-sensitive HCC827 and HCC4006 cells. Treatment-sensitive cells with exosomes secreted by erlotinib-resistant cells induced erlotinib resistance, whereas the knockdown of lncRNA RP11-838 N2.4 aborted this effect. It was further detected that forkhead box protein O1 (FOXO1) was enriched in the promoter region of lncRNA RP11-838N24, leading to its silencing via the recruitment of histone deacetylase. In addition, the high expression levels of serum exosomal lncRNA RP11-838N2.4 in patients displayed resistance to erlotinib treatment [130]. Choi et al. identified exosomal proteins characteristic of getinitib-resistant PC9R cells and found that these proteins mainly originated from membrane-associated components, cytoskeleton, and plasma membrane. Further analysis suggested the AKT/mTOR signaling were correlated with gefitinib resistance. Moreover, exosome-transferred AKT/mTOR complex to

recipient cells was crucial to gefitinib resistance [131]. The expression level of miR-214 was higher in gefitinib-resistant PC9GR cells than gefitinib-sensitive PC9 cells. Exosomes transferred miR-214 from PC9GR cells to PC9 cells, which conferred gefitinib resistance in PC9 cells. PC9GR cell-derived exosomes transfected with miR-214 antagomir reversed gefitinib resistance. Thus, exosomal miR-214 may mediate gefitinib resistance [132]. Lei et al. found that the expression of lncRNA H19 was up-regulated in gefitinib-resistant cells when compared with sensitive parent cells. This further demonstrated that exosomal H19 can induce gefitinib resistance [133]. Altogether, these results reveal an association between EGFR-TKIs resistance and exosomes, which provide a new concept to study EGFR-TKIs resistance, meanwhile, exosomes may be a promising therapeutic target for NSCLC patients with EGFR-TKIs resistance.

Exosomes as diagnostic and prognostic biomarkers in lung cancer

There are no validated techniques for screening of lung cancer other than the low-dose computed tomography (LDCT) [134, 135]. Significantly, liquid biopsy is being developed to serve as a minimally invasive diagnostic and predictive tool for lung cancer [136]. Complement fragment C4d was found to be elevated in biological samples from lung cancer patients, and correlated with poor prognosis, which may be of value for the diagnosis and prognosis of lung cancer at a very early stage [137]. Sozzi et al. demonstrated that plasma microRNA signature classifier (MSC) had predictive, diagnostic, and prognostic value, and MSC may complement LDCT screening via reducing false-positive rates [138]. Montani et al. validated a serum microRNA signature (the miR Test) that might serve as a useful tool for lung cancer screening in a high-risk population [139]. Cohen et al. described a multi-analyte blood test, called cancer SEEK, which could detect both the presence of relatively early cancers and localize the organ of origin of cancers through combined evaluation of the levels of circulating proteins and driver gene mutation [140]. Herein, exosomes represent ideal biomarkers in lung cancer (Table 3), apart from their accessibility and stabilization in most body fluids, which also reflect parental cells signature, as detailed below.

Exosomal microRNA

Exosomal miR-96 was expressed at higher levels in serum from lung cancer patients than normal people. Accordingly, a higher exosomal miR-96 level was positively associated with metastasis and high-grade lung cancer [141]. Exosomal miRNAs, adenocarcinoma (AC)-specific miR-30a-3p, miR-181-5p, miR-361-5p and miR-30e-3p, and squamous cell carcinoma (SCC)-specific miR-15b-5p, miR-320b and miR-10b-5p, were isolated from the plasma of lung cancer patients. These exosomal miRNAs can discriminate between AC and SCC as noninvasive biomarkers for early NSCLC diagnosis [142]. The exosomal miR-21 and miR-4257 levels of the NSCLC patients showed a significant increase in those subjects with recurrence compared with those without recurrence and healthy individuals. Further assessing the clinical significance of these miRNAs, exosomal miR-21 showed a significant correlation with tumor size and tumor node metastasis (TNM) stage, and exosomal miR-4257 showed a significant correlation with lymphatic invasion, histological type and TNM stage. In addition, the high expression levels of exosomal miR-21 and miR-4257 were related to worse survival rate [143]. Rabinowits et al. detected 12 specific 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 the circulating exosomes, suggesting that circulating exosomal miRNA can act as screening markers for lung adenocarcinoma [144]. Exosomal miR-126 was significantly higher in early-stage NSCLC patients than those at advanced stage, showing exosomal miR-126 as a diagnostics biomarker for NSCLC progression [145]. Hydbring et al. investigated exosomal RNA profiling that could distinguish patients with lung adenocarcinoma from benign inflammatory processes. The results revealed that miR-200 family (miR-200b, miR-200c and miR-141) and the mRNA transcript encoding lipocalin-2 (LCN2) were the most substantially up-regulated in the lung adenocarcinoma group, and further suggested miR-200 family (AUC: 0.95) and LCN2 (AUC: 0.9916) as diagnostic markers in lung cancer liquid biopsies [146]. The high level of circulating exosomal miR-222-3p was associated with poor prognosis following gemcitabine therapy in NSCLC patients [147]. Wang et al. identified 9 exosomal miRNAs (miR-141-3p, miR-200a-3p, miR-200b-3p, miR-200c-3p, miR-203a-3p, miR-205-5p, miR-375, miR-429 and miR-483-5p) that were the most abundant in pleural effusion of lung adenocarcinoma when compared to that of tuberculosis or other benign lesions. Furthermore, miR-375, miR-429 and miR-483-5p were validated to be correlated with lung adenocarcinoma [148]. Lai et al. developed a mathematical model to assess exosomal miR-21, miR-205 and miR-155 correlation with tumor volume and showed a combination of exosomal miR-21, miR-205 and miR-155 as diagnostic biomarkers for early screening of NSCLC [149]. Cazzoli et al. analyzed exosomal miRNA from 30 plasma samples, identified 4 microRNAs (miR-378a, miR-139-5p, miR-379 and miR-200b-5p) to discriminate between lung adenocarcinomas and carcinomas. In addition, six microRNAs (miR-30a-3p, miR-151a-5p, miR-200b-5p, miR-100, miR-629 and miR-154-3p) were selected to distinguish lung adenocarcinoma from granuloma [150].

Table 3 Exosomes as diagnostic and prognostic biomarkers in lung cancer

Exosomal cargos	Biofluid	Method	Clinical significance	References
(1) miRNA				
miR-96	Serum	qPCR	High expression was positively associated with metastasis and high-grade lung cancer	[141]
miR-30a-3p, miR-181-5p, miR-361-5p, and miR-30e-3p	Plasma	miRNA-seq	Lung adenocarcinoma -specific miRNA	[142]
miR-15b-5p, miR-320b, and miR-10b-5p	Plasma	miRNA-seq	Lung squamous cell carcinoma-specific miRNA	[142]
miR-21, miR-4257	Plasma	RT-qPCR	High miR-21 and miR-4257 levels predicted recurrence and worse survival rate	[143]
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	Plasma	miRNA microarray	Serve as screening markers for lung adenocarcinoma	[144]
miR-126	Serum	qRT-PCR	High levels associated with early-stage NSCLC patients	[145]
miR-200b, miR-200c and miR-141	Pleural effusions	qRT-PCR	High levels associated with lung adenocarcinoma	[146]
miR-222-3p	Plasma	qRT-PCR	A prognostic biomarker for predicting gemcitabine sensitivity in NSCLC patients	[147]
miR-375, miR-429 and miR-483-5p	Pleural effusion	qRT-PCR	Distinguished lung adenocarcinoma from tuberculous or other benign lesions	[148]
miR-21, miR-205 and miR-155	Serum	A mathematical model	For early detection of NSCLC	[149]
miR-378a, miR-139-5p, miR-379, and miR-200b-5p	Plasma	qRT-PCR	Discriminated between lung adenocarcinomas and carcinomas	[150]
miR-30a-3p, miR-151a-5p, miR-200b-5p, miR-100, miR-629 and miR-154-3p	Plasma	qRT-PCR	Distinguished lung adenocarcinoma from granuloma	[128]
miR-146a-5p	Serum	qRT-PCR	Predicted the efficacy of cisplatin for NSCLC patients and real-time monitoring drug resistance	[151]
miR-30b, miR-30c, miR-103, miR-122, miR-195, miR-203, miR-221, and miR-222	Plasma	qPCR	All correlated with NSCLC	[152]
miR-205-5p, miR-200b	Pleural effusions	RT-PCR	All correlated with lung cancer	[153]
miR-10b-5p, miR-21-5p, and miR-23b-3p	Plasma	qPCR	High levels related to poor overall survival	[154]
miR-425-3p	Serum	qRT-PCR	High levels correlated with platinum-resistant patients	[155]
(2) Proteins				
LBP	Serum	ELISA	Distinguished between patients with metastatic and non-metastatic NSCLC	[156]
CD151, CD171 and TSPAN8	Plasma	EV array	All correlated with lung cancer; high levels of CD151 correlated with SCLC	[157]
CD9, CD81 and CD151	Plasma	EV Array	All correlated with lung cancer	[158]
NY-ESO-1	Plasma	EV Array	Correlated with overall survival	[159]
Tim-3/Galectin-9	Plasma	ELISA	Positively associated with larger tumor size, advanced stages and more lymph node metastasis as well as distant metastasis	[160]
HUWE1, TPM3, SRGN, and THBS1	Plasma	Mass spectrometry	Differentiated lung adenocarcinoma from controls	[161]
LRG1	Urinary	Western blot	High levels correlated with NSCLC	[162]
(3) lncRNA				
MALAT-1	Serum	qRT-PCR	Positively correlated with tumor stage and lymphatic metastasis	[163]

Table 3 (continued)

Exosomal cargos	Biofluid	Method	Clinical significance	References
lncRNA GAS5	Serum	qRT-PCR	Identified patients with early NSCLC	[164]
(4) Lipid				
Lipids	Plasma	UHR-FTMS	Distinguished early- and late-stage NSCLC patients from healthy individuals	[165]

PCR Quantitative real-time polymerase chain reaction, *LBP* lipopolysaccharide-binding proteins, *ELISA* Enzyme-linked immunosorbent assay, *Tim-3* T cell Immunoglobulin- and mucin domain-containing molecule 3, *HUWE1 E3* ubiquitin-protein ligase HECT, UBA and WWE domain-containing protein 1, *TPM3* actin filament-binding protein tropomyosin alpha-3 chain, *SRGN* secretory vesicle proteoglycan serglycin, *THBS1* adhesive glycoprotein thrombospondin-1, *LRG1* the leucine-rich a-2-glycoprotein, *UHR-FTMS* ultra high-resolution fourier transform mass spectrometry, *GAS5* growth arrest-specific transcript 5

The high level of serum exosomal miR-146a-5p showed the chemosensitivity of NSCLC to cisplatin, and reduced recurrence rates. Thus, exosomal miR-146a-5p can be a prognosis biomarker for NSCLC patients to cisplatin response and real-time detection drug resistance [151]. Giallombardo et al. isolated exosomes from 18 plasma samples, identified a panel of exosomal miRNAs (30b, 30c, 122, 103, 203, 195, 222, 221), all associated with NSCLC [152]. Exosomes were isolated from the pleural effusions that were obtained from patients with lung cancer, pulmonary tuberculosis and pneumonia. Exosomal miR-205-5p and miR-200b were significantly increased in lung cancer patients, while exosomal miR-378i was markedly elevated in pulmonary tuberculosis [153]. The high expression of exosomal miR-10b-5p, miR-21-5p and miR-23b-3p was independently related to poor overall survival (OS) [with hazard ratio (95% confidence interval): 2.22 (1.18–4.16), $P=0.013$; 2.12 (1.28–3.49), $P=0.003$; 2.42 (1.45–4.04), $P=0.001$, respectively] [154]. Yuwen et al. analyzed 170 serum samples from advanced NSCLC patients and found that the expression of exosomal miR-425-3p was increased in platinum-resistant patients, which was associated with poor progression-free survival (PFS) [155].

Exosomal proteins

Lipopolysaccharide-binding proteins (LBP) in serum exosomes were discovered to be significantly distinguished between patients with metastatic and non-metastatic NSCLC. Receiver operating characteristic (ROC) curves showed that exosomal LBP had an area under the curve (AUC) of 0.803 with a specificity of 67% and a sensitivity of 83.1% [156]. Exosomes was purified from the plasma of 431 lung cancer patients and 150 controls and tetraspanins CD151, cell adhesion molecule CD171 and TSPAN8 were detected to be significantly up-regulated in the lung cancer patients. However, only CD151 was remarkably up-regulated in the SCLC patients [157]. Jakobsen et al. explored the potential of plasma exosomal protein markers in diagnosing NSCLC. The EV array shows that CD9, CD81 and CD151 were highly expressed in the cancerous patients [158]. Likewise, according to the EV array, NY-ESO-1 showed a strong impact on OS [hazard rate (HR) 1.78, 95% (1.78–2.44); $P=0.0001$] in NSCLC [159]. Gao et al. revealed that exosomal Tim-3/Galectin-9 was positively associated with a larger tumor size, advanced stages and more lymph node metastasis as well as distant metastasis. Additionally, exosomes from lung adenocarcinoma showed a lower Tim-3/Galectin-9 compared to those from lung squamous cell carcinoma [160]. Vykoukal et al. analyzed plasma exosomal proteins from subjects with lung adenocarcinoma and matched controls, and identified four exosome-associated proteins, E3 ubiquitin protein ligase HECT, UBA and WWE domain-containing protein 1 (HUWE1), actin filament-binding protein tropomyosin alpha-3 chain (TPM3), secretory vesicle proteoglycan serglycin (SRGN) and adhesive glycoprotein thrombospondin-1 (THBS1) that differentiated lung adenocarcinoma from controls (AUC: 0.90) [161]. The leucine-rich a-2-glycoprotein (LRG1) was validated to be expressed at higher levels in urinary exosomes from NSCLC patients [162].

Exosomal lncRNA

Zhang et al. isolated exosomes from serum of NSCLC patients and healthy subjects and discovered a lncRNA, named MALAT-1, MALAT-1 was significantly increased in exosomes from NSCLC patients, moreover, the level of exosomal MALAT-1 was positively correlated with tumor stage and lymphatic metastasis [163]. In addition, in vitro studies indicated that serum exosome-derived MALAT-1 facilitated tumor growth and metastasis, and reduced lung cancer cells apoptosis [131]. Li et al. found that long non-coding RNA growth arrest-specific transcript 5 (GAS5) was down-regulated in exosomes from NSCLC patients compared with healthy controls. Furthermore, patients with NSCLC in early stage had a higher Exo-GAS5 expression than advanced-stage NSCLC, thus, Exo-GAS5 may be as a

noninvasive serum-based marker to identify patients with early NSCLC [164].

Exosomal lipid

Fan et al. explored the lipid profiles of plasma exosomes from 39 normal subjects and 91 NSCLC patients (44 early stage and 47 late stage), using least absolute shrinkage and selection operator (LASSO) and random forests (RF) identified exosomal lipids features that successfully distinguished early- and late-stage NSCLC patients from healthy individuals. The area under the receiver operating characteristic curve for early- and late-stage NSCLC versus healthy individuals using the selected lipid features was 0.79 and 0.77 for LASSO and 0.85 and 0.88 for RF, respectively [165].

The role of exosomes in lung cancer therapy

Exosome-based immunotherapy

There are increasing evidences showing that tumor cell-derived exosomes (TEXs) and dendritic cell-derived exosomes (DEXs) can induce specific antitumor immunity. DEXs have the potential to eradicate established tumors in a T-cell-dependent manner and TEX bear neoantigens internalized by DCs which could trigger antitumor T cell response [166, 167]. A549 cells transfected a Rab27a overexpression vector and exosomes were isolated from those displayed high levels of CD9, CD63, Hsp70 and Hsp90. Subsequently, dendritic cells (DCs) incubated with these exosomes expressed higher levels of MHC class II, CD80 and CD86, which significantly enhanced CD4+ T-cell proliferation in vitro. In addition, it was also demonstrated that these exosomes which were injected into BALB/c mice significantly inhibited tumor growth in vivo [168]. Exosomes from CD40 ligand gene-modified 3LL Lewis lung cancer cells were demonstrated to be more immunogenic. Coculturing CD40 exosomes with DCs revealed that CD40L exosomes induced the maturation of DCs and stimulated tumor antigen-specific CD4+ T cell proliferation, triggering a strong protective antitumor immunity in vivo [169].

Most notably, DEX-based phase I and II clinical trials have been performed in NSCLC, indicating the safety and feasibility of the approach, as well as the preference of these nanovesicles to stimulate T cell- and NK cell-based immune responses in patients [167]. Besse et al. performed a phase II clinical trial assessing the clinical benefit of exosomes derived from TLR4L-or interferon (IFN)-γ-maturated DCs (IFN-γ-DEX) loaded with MHC I- and II-restricted cancer antigens as maintenance immunotherapy after first-line chemotherapy in NSCLC. The median PFS was 2.2 months and median OS was 15 months. 32% of patients had stable disease beyond 4 months. Only one patient displayed a grade 3 hepatotoxicity [30]. Morse et al. performed a phase I study to evaluate the safety, feasibility and efficacy of autologous DC-derived exosomes (DEX) loaded with the MAGE tumor antigens in patients with advanced NSCLC. DEX therapy was well tolerated and some patients had a relatively long PFS and MAGE-specific T cell responses was detected in 3/9 patients [29]. DEX-based phase I and II clinical trials were discovered to be well tolerated in lung cancer patients, yet, because of the small sample, the data lack credibility, so more extensive clinical trials need to be conducted.

Exosomes as anticancer drug delivery vehicles

There are emerging evidences showing that exosomes is a promising drug delivery system, because exosomes have unique characteristics, for instance, low immunogenicity, decreased toxic, high biocompatibility, and surmount biological barriers (including blood–brain barrier), nanoscale size and target homing specificity [170, 171]. Sun et al. reported a novel nanoparticle drug delivery system, exosome–curcumin complexes, and showed that exosomes can increase solubility, stability, and bioavailability of curcumin, thus enhancing the anti-inflammatory activity of curcumin [172]. Maguire et al. demonstrated that microvesicle-associated adeno-associated virus (AAV) vector as a novel gene delivery system could enhance gene transfer in culture cells [173]. Mizrak et al. evaluated that MVs can act as a cell-derived gene delivery vehicle transferring therapeutic mRNA/protein to target tumor cells, which inhibited schwannoma tumor growth through expressing a high level of functional protein in recipient cells [174]. Tian et al. validated a doxorubicin delivery platform using exosomes that showed highly antitumor activity both in vitro and in vivo [175]. In addition, Kim et al. assessed the feasibility of an exosome-based paclitaxel (PTX) formulation treating multiple drug resistance (MDR) cancer. The result showed that the incorporation of PTX into exosomes significantly increased drug accumulation levels and cytotoxicity in resistant cells. Furthermore, Kim et al. demonstrated that airway administered exosomes arrived to pulmonary metastases and transported their drug payload to target cancer cells in vivo [176]. Srivastava et al. developed an exosome-based drug delivery system for lung cancer therapy, called nanosomes, which consists of GNPs conjugated to anticancer drug doxorubicin (Dox) via a pH-sensitive hydrazone linker loaded onto the exosomes (Exo-GNP-Dox). The increased rate of drug release under acidic conditions, successful uptake of the nanosomes by the recipient cells and the cell viability analysis indicated that nanosomes displayed preferential cytotoxicity to cancer cells other than non-cancerous cells [31]. Celastrol, a plant-derived triterpenoid, suppressed

Table 4 Current clinical trials on exosomes in lung cancer

Title	Status	Conditions	Interventions
Combined diagnosis of CT and exosome in early lung cancer	Not yet recruiting	Early lung cancer	Procedure: surgery
Serum exosomal long noncoding RNAs potential biomarkers for lung cancer diagnosis	Recruiting	Lung cancer	Diagnostic test: collect samples
Clinical research for the consistency analysis of PD-L1 in lung cancer tissue and plasma exosome before and after radiotherapy	Unknown	NSCLC	Radiation: radiotherapy
Dynamic monitoring circulating tumor DNA in surgical patients with lung cancer	Recruiting	Lung cancer	Other: blood samples
Detection of circulating biomarkers of immunogenic cell death	Active, not recruiting	NSCLC	Other: blood samples
Circulating exosome RNA in lung metastases of primary high grade	Recruiting	Lung metastases	
Osteosarcoma	Other: blood samples		
Olmutinib trial in T790 M (+) NSCLC patients detected by liquid biopsy using BALF extracellular vesicular DNA	Active, not recruiting	NSCLC	Drug: olmutinib
Detection of either the EML4-ALK gene rearrangements or the T790 M EGFR mutation in the plasma of advanced NSCLC patients	Active, not recruiting	Carcinoma	
NSCLC	Other: blood samples		
Pilot study with the aim to quantify a stress protein in the blood and in the urine for the monitoring and early diagnosis of malignant solid tumor	Recruiting	Cancer	Other: blood samples
Other: urine samples			

the proliferation of A549 and H1299 cells by its repressive effects on NF-κB activation and cell cycle progression and also through the induction of chronic endoplasmic reticulum stress-mediated apoptosis. Encapsulation of celastrol into bovine milk-derived exosomes showed greater antitumor efficacy and reduced toxic side effects compared to free celastrol [32]. Kim et al. developed an exosome-based drug delivery system AA-PEG-exoPTX that constitutes paclitaxel (PTX)-loaded macrophage-derived exosomes (exo) with incorporated aminoethylanisamide-polyethylene glycol (AA-PEG) vector moiety. It was further demonstrated that AA-PEG-exoPTX AA-PEG-exoPTX efficiently inhibited lung metastases through targeting the sigma receptor that was overexpressed in lung cancer cells [33]. These data supported that exosome-based drug formulations can provide powerful and low immunogenic profile delivery systems for anticancer therapy, however, there are some technological, functional and safe issues that remain unresolved, such as preferential accumulation in cancer cells, efficient transportation of incorporated cargo into target cancer cells and controlled drug release, so more researches are necessary to unveil these mechanisms.

Conclusion

In summary, exosomes can influence lung cancer progression, and exosomes may be an alternative option for lung cancer therapy. Nevertheless, some problems are yet to be addressed. First, bulk extraction and thorough characterization of exosomes remain challenging, which will influence clinical applications, biomedical investigation as well as the production cost, thus, we could combine multiple separation technologies to optimize exosomes isolation and enrichment. Second, exosomes can transfer biologically active molecules between lung cancer cells and their microenvironment (Fig. 2 and Table 2), but, the mechanisms exosomes use to exactly deliver certain cargos to specific target cells need to be illuminated. Third, exosomes are a promising biomarker for lung cancer diagnosis, prediction and prognosis as well as real-time monitoring therapy response (Table 3). However, these studies are generally found with small samples and poor repeatability, so large multicenter studies are necessary to develop effectiveness of liquid biopsy. Finally, the exosome-based clinical trials have been accomplished in lung cancer, moreover, there are still some clinical trials going on (Table 4) and such studies can provide proof for the transition of therapeutics that are based on exosomes into the clinic in the not-so-distant future (Fig. 3).

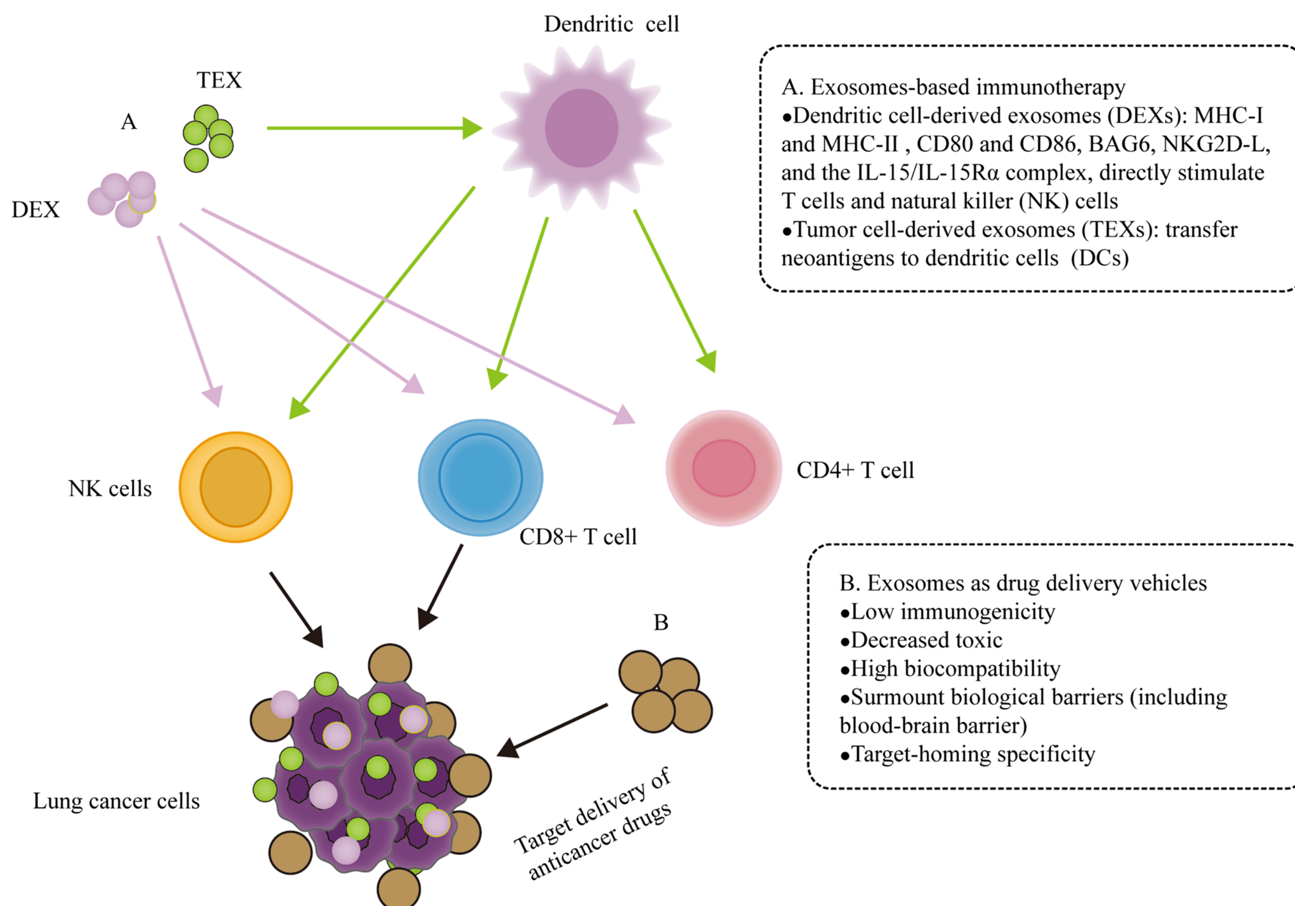

Fig. 3 The role of exosomes in lung cancer therapy. **a.** Tumor cell-derived exosomes (TEXs) and dendritic cell-derived exosomes (DEXs) can induce specific antitumor immunity. TEXs carry neoantigens and deliver immunosuppressive cargos to dendritic cells (DCs), which influence the development, maturation and antitumor activities of immune cells. The presence of MHC I, MHC II molecules and costimulatory molecules (CD80 and CD86) as well as other molecules on the surface of DEX makes them directly stimulate T cells and natural killer (NK) cells, triggering a strong immunity response [166, 167]. **b.** Exosome-based drug vehicles have unique characteristics, such as low immunogenicity, decreased toxic, high biocompatibility, surmount biological barriers and target homing specificity, which make them provide powerful and low immunogenic profile delivery systems for anticancer therapy [170, 171]

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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